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## THE STOMACH SPIROCHETE OCCURRING IN MAMMALS

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In 1893 Bizzozero discovered a spirochete in the stomach of dogs in Italy which he stated was in the parietal cells of the peptic glands. Salomon (1896), examining various animals in Germany, detected the same organism in the stomach of dogs, cats and rats, and was able to transmit it by feeding to the stomach of the mouse. He distinguished three forms morphologically and also found the spirochete to be actively motile with terminal flagella. Balfour (1906), in Egypt, observed spirochetes in gastric and intestinal ulcers of dogs and monkeys, produced by inoculation with a trypanosome (*Trypanosoma dimorphon?*). In the same year Krienitz (1906), in Germany, found three forms of spirochetes in the fresh stomach contents of a patient suffering from stomach cancer and later (1906a) studied the morphological changes resulting from alternations in environment. Afterward Regaud (1909), in France, found the organism by means of darkfield illumination and proved it to be a living micro-organism, notwithstanding that Carnot and Lelièvre (1909) had described it as the secretion product of parietal cells. In the following year Lucet (1910) detected two forms of spirochete in lesions of a dog suffering from hemorrhagic gastro-enteritis. Ball and Roquet (1911), however, regarded this spirochete as identical with that described by Regaud and called it *Spirochaeta regaudi*. They stated, moreover, that this spirochete occurring in the normal stomach of dogs has probably no causative relation to hemorrhagic gastro-enteritis. Suda (1916), in Japan, also observed spirochetes in the gastric gland of dogs.

The present paper is a report of investigations undertaken in connection with our encountering this spirochete in the stomach of a rat in the course of an examination of the alimentary tract (1917).

*Morphology.*—The stomach spirochete is an inflexible spiral with a straight longitudinal axis, the transverse section being an ellipse, and is provided with one short, thin flagellum at each end. In the stained preparation, the most common type of this spirochete (Fig. 1) is a fine form measuring from 4.5 to 10.5 $\mu$  and the number of turns is 4 or 5 to

14 or 15. Every two consecutive turns are closely set, the distances between these pairs of spirals being regular. There was frequently seen, however, in the stomach of the cat and monkey a form somewhat shorter, with a wider spiral and a more acute turn (Fig. 2). Another form (Fig. 3), usually occurring in the stomach of the dog, is stout, and the turns are shallower and fewer. Such a form is especially observed in the stomach of the rabbit, and also often of the mouse and guinea-pig, if it be transmitted.

Thus, like Salomon, we were able to distinguish three forms morphologically, although our classification is slightly different. It is uncertain whether these forms represent in reality three different species or belong to one species with temporary variations in form. The degenerated spirochete frequently assumes the spiral body curved or twisted, or with irregularly relaxed turns. There are also seen individuals faintly or heterogeneously stained, and even in "moniliform degeneration" (Fig. 1). Moreover, in preparations stained with Giemsa's stain, we can very often detect so-called "involution forms," which show from one to five or more chromatin-like granules stained intensely red at the outer ends of turns, notwithstanding the faint staining of the body. Such granules also are occasionally detected arranged along the main axis in the rod-shaped spirochete (Fig. 3).

This spirochete in the supravital staining is not found differentiated from that of the foregoing description.

The spirochete in the dark-field illumination shows a fair distinction (Fig. 4). The consecutive turns seem almost to touch, and accordingly the whole body presents the appearance of a coil, the transverse section of which was clearly proved by the rotatory movement of the organism to be an ellipse. The dark-field microscopic view also shows that each of the two extremities of this spirochete is tapered into a fine terminal flagellum.

*Staining.*—Compared with the other spirochetes, the spirochete under discussion takes stain very readily with the basic anilin dyes, viz., fuchsin, methylene blue, gentian violet, etc. For the staining of smears, however, Manson's borax methylene blue, Giemsa's stain and Fontana-Tribondeau's method are especially to be recommended. For the staining of the organisms in tissues, iron-hematoxylin staining is superior to Levaditi's silver impregnation. Here Levaditi's method, although it is convenient for proving the existence of terminal flagella, is not suitable for differentiating the spirochetes in the stomach glands (Fig. 5). This is probably due to the mucus, which surrounds the organism and perhaps prevents its impregnation with silver.

As the relief staining, Benians' (1916) method is not only simple, but gives an excellent preparation (Fig. 6). The procedure is as follows: A small drop of a 2 per cent. aqueous solution of Congo red

is placed on a slide and a very small quantity of the material to be examined is rubbed into it with the platinum loop. The drop is then spread out into a rather thick film and allowed to dry. The slide is then washed over with a 1 per cent. solution of HCl in absolute alcohol and dried in the air. The film is then ready for examination.

*Movement.*—The movement of this spirochete is comparatively rapid and very simple. Examination under the dark-field microscope shows that the organism moves only forward and backward inflexibly in a straight line, and progression always takes place by the vibration of the posterior flagellum. Occasionally, however, there were observed a rotatory movement around the long axis, a snakelike movement, a movement forming the outline of a cone with a fixed end as apex, etc.

It must be remembered that, for examination by dark-field illumination, the material to be examined must, in most cases, be diluted with a few drops of water, otherwise the free movement of the organism is decidedly limited by compression of the tissue mass.

*Distribution Among Animals.*—We examined the stomachs of thirteen monkeys, forty-nine dogs, thirteen cats, twenty rabbits, fifteen guinea-pigs, thirty-eight wild rats, ten white rats, fifteen mice and fifteen field voles. All of the specimens examined were fresh, the animals having been killed only a short time before examination, except in the cases of six dead dogs and four cats, which, however, were examined shortly after death. The result of our examination is as follows:

1. The spirochete was detected in forty-three out of forty-nine dogs. Five out of six negative cases, however, were young from the same mother, only two or three weeks after weaning.
2. Out of thirteen cats eight gave a positive result, and the five negative cases were all very young.
3. Thirty-eight wild rats yielded only one positive case (*Epimys rattus alexandrinus*).
4. Thirteen monkeys were all positive.
5. Among rabbits (inoculated with *virus fixe* of rabies), guinea-pigs, white rats, mice and field voles, there was no positive case.

Judging by the foregoing results, the invasion of this spirochete seems to have a close relation to the life condition of the host. It was detected in nearly all cases of adult dogs and cats, which wander from place to place, devouring whatever food they happen to find. One hundred per cent. of the monkeys in which the spirochete is found show an extensive variation of diet. The limited life, on the other hand, may explain the rare occurrence of this organism among young dogs, young cats and wild rats, and its non-occurrence among other experimental animals, such as white rats and mice.

*Distribution in the Animal Body.*—We looked for the spirochete in various parts of the alimentary canals of twenty-six animals, naturally or experimentally infected; i. e., five dogs, two cats, three wild rats, five white rats, six mice and five rabbits. While the organism was always detected abundantly in the stomach of all these, in the mouth cavity and cecum, no similar spirochete could be found in any animal. But a few degenerated specimens were detected in the esophagus of four dogs, one cat, one wild rat, one white rat, one mouse and three rabbits, and still fewer in the duodenum of one dog, one white rat and one mouse. Moreover, the stomach contents were examined in four dogs, one cat, two white rats, one wild rat, one mouse and five rabbits, and only a few degenerated specimens were detected there in three dogs, one white rat and three rabbits.

These experiments indicate that the domicile of this spirochete is the stomach. Histological examination also shows that it is principally detected in the fundus gland, especially in its neck, where the organisms arranged parallel to the axis of the duct occasionally swarm so densely as to obstruct the canal. Moreover, organisms were seen lying between the chief cells or even in the cytoplasm of the parietal cells. In the case of dogs and cats, spirochetes were eventually found in the pyloric gland.

*Transmission Experiment.*—Mice, white rats, guinea-pigs and rabbits were selected as experimental animals. In this experiment, about five mice, two or three white rats, two or three guinea-pigs, and usually two rabbits were used for transmission from generation to generation. Here the canine strain was principally used, but occasionally the feline or the monkey strain was employed. These strains gave almost the same result.

To transplant the original strain to experimental animals of the first generation, we scraped the mucous membrane containing large quantities of this spirochete from the stomach of a dog, and fed to mice and rats in small portions and to guinea-pigs and rabbits in large amounts. After the second generation, in the case of mice and rats, a piece of the stomach wall was given to each animal of subsequent generations, and guinea-pigs and rabbits were fed a large quantity of the finely crushed mucous membrane.

Following are the results of the transmission experiment:

1. In the case of mice, we obtained the most satisfactory result, distinct multiplication being observed as early as the second day after transmission. The procedure was continued for fifteen generations, and it was found that there was a remarkable increase in every generation.

2. The transmission was also very easy in the case of white rats and the experiment was therefore discontinued after the tenth genera-

tion. The same result was obtained in the case of wild rats, where passage was continued until the fifth generation.

3. In the case of normal guinea-pigs, the transmission was very difficult. The first experiment was continued with difficulty until the third generation; by making use of animals infected by scarlet fever or measles, however, we were easily able to continue it until the tenth generation.

4. In the case of normal rabbits, we were unable to carry the procedure through the fifth generation. If the animal infected with this spirochete be inoculated with *virus fixe* of rabies, however, transmission becomes extraordinarily easy. The canine strain was thus passed without difficulty through ten generations and the feline through five generations.

#### EXPERIMENTS ON RESISTANCE

##### I. LYTIC ACTION OF SAPONIN, SODIUM TAUROCHOLATE AND BILE

The material used for the experiment was the feline strain. The mucous membrane, in which large numbers of spirochetes had been detected, was scraped from the stomach of a cat immediately after death, and diluted with saline solution. Saponin and sodium taurocholate were used in 10 per cent. aqueous solution. The bile was obtained from the cat and used without being diluted.

The procedure was as follows: Equal quantities of each of these chemicals and the spirochete-containing suspension were thoroughly mixed and, at required intervals, a small drop of it was spread upon a slide by means of platinum loop. It was then dried above a weak flame (about two minutes), fixed with methyl alcohol for fifteen minutes and stained by Manson's stain under as nearly the same conditions as possible.

The result of this experiment is recorded in Table 1.

TABLE 1.—LYTIC ACTIONS OF SAPONIN, SODIUM TAUROCHOLATE AND BILE ON THE SPIROCHETE

Chemicals	15 Mins.	30 Mins.	1 Hr.	2 Hrs.	3 Hrs.
Saponin	Spirochetes became swelled and stained unfavorably. Some in process of dissolution	Staining very faint, and spirals irregular and indistinct	Almost complete dissolution	Almost complete dissolution	A very few degenerated organisms remaining
Sodium taurocholate	A few degenerated spirochetes remaining	Complete dissolution			
Bile	A few spirochetes in process of dissolution	Degenerated forms unlike the spirochete seen very rarely	Complete dissolution		
Control	No change	Degenerated forms unlike the spirochete seen very rarely	Complete dissolution	Almost complete dissolution	A very few degenerated organisms remaining

## II. RESISTANCE OF THE SPIROCHETE AGAINST PUTREFACTION

(a) *Experiment in the Refrigerator.*—This experiment was made in July and August. The contents having been removed, the stomach wall containing spirochetes was placed in the refrigerator (8 to 10° C.). Every other day some of the mucous membrane was scraped off and fed to two mice, to ascertain whether it still contained live spirochetes. In three specimens from the dog and in two from the cat the results indicated that the spirochete under discussion generally continues its life in such condition for about ten days (from seven to fourteen days), showing that the death of this organism has a close relation to the putrefaction of the stomach wall. If after ten days the stomach wall putrefies to any degree and spirochetes are no longer perceived under the microscope, transmission to mice generally becomes impossible.

(b) *Experiment in a Room.*—This experiment was performed in August. The materials obtained from one dog and three mice, all heavily infected, were exposed in a room (average 30° C. in the former case and 28° C. in the latter) for twenty-four hours and the putrefied material given to two mice. Except in the case of one mouse, the result was negative. If the contents be allowed to remain in the stomach, however, this spirochete seems to disappear more quickly. We observed that the spirochete in question disappeared within about ten hours in such a stomach, even in the refrigerator. The result is probably due to the lytic action of split products of the stomach contents.

## III. ACTION OF SALVARSAN ON THE SPIROCHETE IN VIVO

(a) *Infusion of Salvarsan Into the Infected Stomach.*—We selected as the experimental animals mice previously infected with large numbers of spirochetes. Arsaminol (salvarsan made in Japan) was used as an acid solution diluted only with saline solution, viz., 1:100, 1:200, 1:300, 1:500, 1:1,000 and 1:2,000. The solution was introduced directly into the stomach cavity of the mouse (1 c.c. per cap.) by means of catheter at the time of starvation. Twenty-four hours later the mice were killed and the mucous membrane of the stomachs examined under the dark-field microscope.

The result is indicated in the following table:

TABLE 2.—STERILIZATION EFFECTS OF ACID SALVARSAN SOLUTION ON THE SPIROCHETE IN VIVO

Mouse Number	Degree of Dilution of Salvarsan	Spirochete
1, 2, 3	1:100	—
4, 5, 6	1:200	—
7, 8, 9	1:300	—
10, 11	1:500	+
12, 13	1:1000	+
14, 15	1:2000	+
16, 17, 18, 19	Control	+

N.B.—Mouse 9 died about 15 minutes after infusion. Examination of the stomach revealed no spirochetes.

The table shows that the 1:300 acid solution of salvarsan can still sterilize the spirochete in the stomach of the mouse.

(b) *Intravenous Injection of Neosalvarsan*.—The 1:200 solution of neoarsaminol (neosalvarsan made in Japan) was intravenously injected into the five spirochete-bearing mice to the amount of 0.05 c.c. for 1 g. of weight, viz., in the maximum dose. Thirty hours later the mice were killed and their stomachs examined by dark-field illumination. The stomachs showed the same negative result as the controls (two normal mice).

*Pathogenicity*.—If the host is normal, occurrence of this spirochete in the stomach exerts no pathogenicity. If the spirochete-bearing rabbit be inoculated with the *virus fixe* of rabies, however, the spirochete abundantly increases in number and causes a specific lesion in the stomach of the host.

Such rabbits, showing rabid symptoms a week after the inoculation of the *virus fixe*, were killed, and on examination the stomach usually contained only fluid with no food particles. A large quantity of mucus always covered the surface of the mucous membrane. Marked hyperemia and hypertrophy of the mucosa, especially in the fundus, were also present. In such cases, *punctate hemorrhages, even the so-called hemorrhagic erosions, were constantly detected on both sides near the middle along the greater curvature*. Upon histological examination, the hyperemic and hemorrhagic areas were found to be located principally in the mucosa, especially in the free end of the glandular layer, but frequently also in the submucosa. The spirochetes were always abundantly detected in the lesions, where they appeared not only in the ducts of glands but sometimes even in the tissue, while they were rarely, if ever, found in the apparently normal parts. No such remarkable lesion has ever been seen in the stomach of the rabbits infected only with the *virus fixe*.

Tables 3 and 4 show the results obtained with the canine and feline strains. They also show that if, a certain interval after infection of this spirochete, the rabbit be inoculated with the *virus fixe*, the autopsy performed one week later will show that abundant increase of the spirochetes causes a severe hemorrhagic gastritis in the host. It is concluded that the infection with the *virus fixe* probably causes a gastric disturbance apparently as invisible as a very slight catarrhalic gastritis in the rabbit, and that a stomach so affected becomes a favorable medium for this spirochete. Then large increase in the number of spirochetes seems to cause the slight primary disturbance secondary to the heavy hemorrhagic gastritis. The reason for this secondary pathogenicity, however, is still uncertain. The same experiment was repeated on ten mice, and only three cases of the slight hemorrhagic gastritis were detected.

TABLE 3.—RESULT OBTAINED BY THE INOCULATION OF THE VIRUS FIXE INTO THE CANINE-STRAIN BEARING RABBITS

Rabbit No.	Generation	Date of Transmission of Spirochetes	Date of Inoculation of the Virus Fixe	Interval between Transmission and Inoculation	Occurrence of Spirochetes	Lesion of the Gastric Mucosa
1	I	May 28, 1917	May 28, 1917	0	++	—
2	I	May 28, 1917	May 28, 1917	0	—	—
3	I	May 28, 1917	May 28, 1917	0	—	—
4	I	May 28, 1917	May 29, 1917	1	—	—
5	II	June 4, 1917	June 7, 1917	3	+++	+
6	II	June 4, 1917	June 7, 1917	3	+++	++
7	III	June 14, 1917	June 18, 1917	4	+++	+++
8	III	June 14, 1917	June 18, 1917	4	+++	+++
9	IV	June 25, 1917	June 30, 1917	5	+++	+++
10	IV	June 25, 1917	June 30, 1917	5	+++	++
11	V	July 7, 1917	July 9, 1917	2	++	—
12	V	July 7, 1917	.....	...	—	—
13	VI	July 16, 1917	July 22, 1917	6	?	+++

TABLE 4.—RESULT OBTAINED BY THE INOCULATION OF THE VIRUS FIXE INTO THE FELINE-STRAIN BEARING RABBITS

Rabbit No.	Generation	Date of Transmission of Spirochetes	Date of Inoculation of the Virus Fixe	Interval between Transmission and Inoculation	Occurrence of Spirochetes	Lesion of the Gastric Mucosa
14	I	July 9, 1917	.....	...	—	—
15	II	July 19, 1917	July 23, 1917	4	+++	+++
16	III	July 30, 1917	Aug. 7, 1917	8	+++	+++
17	IV	Aug. 14, 1917	Aug. 20, 1917	6	+++	+++
18	IV	Aug. 14, 1917	Aug. 20, 1917	6	+++	+++
19	V	Aug. 27, 1917	Aug. 29, 1917	2	+++	++
20	V	Aug. 27, 1917	Aug. 31, 1917	4	+++	++
21	V	Aug. 27, 1917	Sept. 1, 1917	5	+++	+++
22	V	Aug. 27, 1917	Sept. 3, 1917	7	+++	+++
23	VI	Sept. 5, 1917	Sept. 6, 1917	1	+++	+
24	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++
25	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++
26	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++

N. B.—(1) Under "Occurrence of spirochetes": — indicates negative result; + from one to several spirochetes in a preparation; ++ from one to several in several fields; +++ several or more in a field.

(2) Under "Lesion of the gastric mucosa": — indicates the apparent absence of lesions; + hyperemia and hypertrophy moderate, hemorrhage very slight, gastric contents juicy and mucus abundant; ++ hyperemia and hypertrophy distinct, hemorrhage moderate and contents fluid, with only a small quantity of floating solid particles; +++ hemorrhage remarkable and contents completely fluid.

(3). Rabbits 12 and 14 are controls, which were only subjected to the inoculations of the *virus fixe*.

(4). Rabbit 13 died in the early morning on the day on which we intended to kill it; its stomach with the contents was immediately placed in the refrigerator. About ten hours later, upon examination of the stomach, no spirochetes could be detected, while marked hemorrhage was observed. This shows that, in all probability, the spirochetes were dissolved by the split products of the stomach contents.

Moreover, the stomachs of guinea-pigs previously infected with measles or scarlet fever and fed with the spirochetes constantly showed a great increase of the spirochete and the distinct hyperemia and hemorrhage of the mucosa.

The conclusion may be drawn from these results that the cases of hemorrhagic gastro-enteritis described by Balfour and Lucet are in all probability due to the secondary pathogenicity of this spirochete.

As a sequel to the foregoing experiment, we inoculated the emulsion of the gastric mucosa, containing large numbers of this spirochete, into the testes of four white rats, but no multiplication of the organism was found to have occurred.

#### SUMMARY

1. The stomach spirochete is an inflexible spiral with a straight longitudinal axis, the transverse section being an ellipse. It is provided with a flagellum at each end.

2. It takes stain very readily, compared with the other spirochetes, not only by the basic anilin dyes commonly used for the staining of bacteria, but by iron hematoxylin.

3. Its movement is comparatively active and very simple, progression being only forward and backward in a straight line.

4. This organism was detected in forty-three out of forty-nine dogs, in eight out of thirteen cats, in one out of thirty-eight wild rats and in every one of thirteen monkeys, but was absent in twenty rabbits, fifteen guinea-pigs, ten white rats, fifteen mice and fifteen field voles.

5. Its domicile is the stomach, especially the fundus gland.

6. It is readily soluble in saponin, sodium taurocholate and bile. It is also labile to putrefaction.

7. The introduction of salvarsan into the stomach is easily capable of sterilizing the spirochetes domiciling there.

8. The organism is readily transmitted to the stomach of the rat or mouse, but transmission to the normal rabbit or guinea-pig is very difficult.

9. If, after a certain interval, a rabbit previously infected with the spirochete be again inoculated with the *virus fixe*, the stomach of the host, at autopsy performed a week after inoculation, shows a distinct increase of spirochetes and a remarkable hemorrhagic inflammation in the mucosa. The same result was obtained in guinea-pigs previously infected with scarlet fever or measles after subsequent feeding with this spirochete.

We wish to express here our deep indebtedness to Prof. S. Kitasato, director of the Kitasato Institute, and to Profs. S. Hata and S. Kusama for their cordial guidance.

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## DESCRIPTION OF PLATE

Fig. 1-3.—Three types of the spirochete.

Fig. 4.—Various forms of the spirochete under the dark-field microscope.

Fig. 5.—Various forms of the spirochete stained with Levaditi's method.

Fig. 6.—Figures of the spirochete treated with Benians' relief staining.

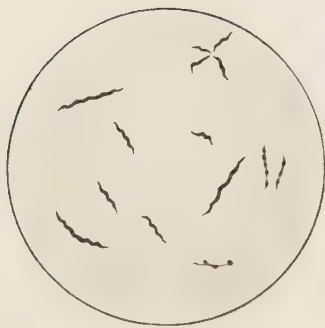
KASAI-KOBAYASHI—STOMACH SPIROCHETE



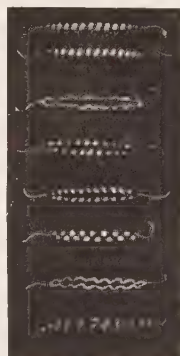
1



2



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4



5



6



ON THE SPECIFIC IDENTITY OF *HERONIMUS*  
*CHELYDRAE* MACCALLUM AND *AORCHIS*  
*EXTENSUS* BARKER AND PARSONS

HORACE W. STUNKARD

The monostomes are among the least known of North American trematode groups. Records give descriptions of only six species, each the single representative of a genus, and according to the classification of Ward (1918) belonging to four different families. Existing descriptions in most cases are far from complete and data necessary for taxonomic determination are lacking. This deficiency has been pointed out by other workers, both in this country and in Europe, and the classification of the monostomes is not well established. In fact, certain investigators regard them as aberrant forms, sprung from different distome groups, which alike have lost the acetabulum. If this is true and the similarity is merely superficial, the present system of classification must be entirely revised. Careful, complete descriptions of these forms are necessary to provide the data for a natural system of classification. The present study, it is hoped, will prove a step toward the solution of this problem.

*Heronimus chelydrae* was described by MacCallum (1902) from the lungs of the American snapping turtle, *Chelydra serpentina*, taken at Ontario, Canada. After the description of the form, the author stated, "It seems necessary, therefore, to establish a new genus in the family Monostomidae to accommodate this form—a genus which stands far apart from the other genera in several respects, but especially in the position and nature of the genital opening, in the complicated structure and course of the uterine tract, the unusual formation of the yolk glands, in the presence of but one testicle, and in the position of the excretory pore."

Barker and Parsons (1914) in a preliminary announcement described a monostome, parasitic in the lungs of *Chrysemys marginata*, which they named *Aorchis extensus*. In a later paper (Barker and Parsons, 1917) they gave a more extended description of the form based on the study of the specimens originally secured from Lake Emily, Minnesota, and others found later in the lungs of the same host taken from the Mississippi River near Fairport, Iowa. As diagnostic characters of the genus *Aorchis* they stated:

"Body medium to large, slightly tapering toward the anterior and posterior ends, posterior end rounded. Oral sucker small, weak but distinct. Mouth opening terminal. Pharynx strongly muscular, with-

out pockets. Esophagus short. Intestine composed of two simple blind sacs, not uniting at the posterior end. Genital pore not prominent, ventral to the pharynx, close to oral sucker. Ovary anterior between intestinal ceca. Shell gland compact, posterior to and smaller than ovary. Uterus made up of coils which fill the body lateral to and overlap the intestinal ceca, extending from level of the ovary to posterior end of the body and two straight and parallel uterine tubes which pass anteriorly up the median axis of the body between the intestinal ceca. Vitelline gland a voluminous, coarse, compact, U-shaped mass posterior to ovary and dorsal to the intestine, with the closed portion at the anterior end. Protandrous. Testis absent, or atrophied in old worms. A single ovoidal testis present in young worms, anterior, caudad to ovary. Prostate gland near testis, seminal vesicle, a large tubular structure extending from genital pore to the level of the second anterior fifth of the body. Protrusible nonmuscular cirrus present. Laurer's canal and seminal receptacle absent. Eggs without lids. Excretory pore posterior dorsal."

A new family, Heronimidae, was created by Ward (1917) to contain the two genera *Heronimus* and *Aorchis*. This author had collected specimens which he stated probably belonged to the species *A. extensus*, from the lungs of various turtles from Michigan, Indiana, Illinois and Nebraska. Comparing the two genera he says, "These two forms are so much alike that they may prove to be identical, or at least to belong to the same genus, but they are in some respects very different from any other monostomes known, and I have established for them a new family with the following characters:

"Heronimidae Ward. Moderate sized monostomes with thick, elongate, soft body, slightly flattened, tapering toward both ends. Oral sucker weak, pharynx large, esophagus short or absent; ceca simple, narrow, extending to posterior tip but not united. Vitellaria compact tubular; uterus with four longitudinal regions; genital pore ventral to oral sucker, near anterior tip. Testis tubular, small, copulatory apparatus poorly developed. In lungs of turtles, northern North America."

The following year, Ward (1918) restated the family characters with the following additions: "Vitellaria compact tubular, shaped like an inverted V. Testes tubular, lobed or with short branches, united into a V-shaped organ with the apex anterior. . . . Two genera imperfectly known which may prove to belong in the same genus." The genera *Heronimus* and *Aorchis* he distinguished as follows:

"Vitellaria extend only half way from ovary to posterior end. Seminal receptacle present . . . *Heronimus* MacCallum 1902.

"Vitellaria extend from ovary to posterior end of body. Seminal receptacle absent. . . . *Aorchis* Barker and Parsons 1914."

Ward (1918) gave two figures of *A. extensus* and a brief specific description. He differed from Barker and Parsons with regard to the testis. Ward found "Testes elongate, tubular, irregularly lobed."

While engaged for several years in the study of animal parasites, I have examined about three hundred turtles and found a large monostome in the lungs of six different species collected from the central and southern as well as northern districts of North America. This parasite has been secured from *Chelydra serpentina* taken in Iowa, Illinois, Ohio, North Carolina and Texas. Specimens have been found in the lungs of *Chrysemys marginata* taken in Iowa, Illinois, Missouri and Kentucky; *Pseudemys elegans* and *Malacoclemmys geographicus* in Illinois; *Aromochelys odoratus* and *Kinosternum pennsylvanicum* in North Carolina.

This form I had regarded as identical with *A. extensus* Barker and Parsons and the close similarity to *H. chelydrae* had been noted. Through the kindness of the director of the U. S. National Museum, I have had an opportunity recently to study the specimens of *Heronimus chelydrae* deposited there by MacCallum. In addition, a large specimen of *H. chelydrae* from the collection of Albert Hassall has been placed at my disposal. This worm is from the lung of *Kinosternum pennsylvanicum* taken near Baltimore, Maryland. I wish to express here my thanks to these workers for their kind assistance.

A careful examination of the specimens and comparison with the description of MacCallum confirms his observations. He gave a careful description of the morphology of the parasite and a detailed histological description of certain structures. He described the uterus as extensively convoluted and folded, traversing the length of the body four times, but he did not describe the definite course of the tube and his figure gives a diagrammatic representation rather than a precise picture of the position of the loops and coils of the uterus. Further, he did not describe the extent of the testis or state that in certain specimens this organ is reduced or degenerate.

Comparison of the monostomes I have collected with the type specimen and other sectioned individuals of *H. chelydrae* shows fundamental and precise agreement in every respect and demonstrates that they belong to that species.

A careful study of the material of *H. chelydrae* yields results strikingly different from those of Barker and Parsons. The difference in certain features is so marked that I may be dealing with another species, but in other respects there is such precise agreement that I am inclined to believe I have the same form. Some of the specimens at hand are from the same host and the same locality where Barker and Parsons' material was collected. They agree with the description of Barker and Parsons in size and shape, size and character of the oral

sucker, pharynx, esophagus and digestive ceca, extent and position of the uterine coils, size and position of the ovary, character of the copulatory organs, and location of the genital pore. But in *H. chelydrae* the excretory system is different from that described by Barker and Parsons, the pore is near the anterior instead of the posterior end of the body, the vitellaria are ventral and not dorsal, a seminal receptacle is present, also a V-shaped testis which corresponds in size and extent with Barker and Parsons' description of the vitellaria. If these differences were minor in character, I should conclude that the specimens belong to a different species, but the form of the excretory system is a fundamental and characteristic feature of large groups, and questions that involve the dorso-ventral axis or concern the form of the testis and vitellaria are not of specific nature. Consequently, in view of the agreement in other respects, I am inclined to question the accuracy of Barker and Parsons' description. In their first report (Barker and Parsons, 1914) the eggs are described as possessing a short polar stalk and a statement is made that the cirrus is lacking. Their later paper (1917) does not refer to any polar stalk on the eggs and a cirrus is described. Ward (1918) has made further corrections to the description. The comparison of *H. chelydrae* with the description of *Aorchis extensus* leaves little if any doubt that the two forms are identical. Barker and Parsons do not refer to the work of MacCallum and have not published a comparison of their form with *H. chelydrae*. The unsatisfactory nature of Barker and Parsons' description and the agreement of the parasites described by them with *Heronimus chelydrae* discredits the validity of the genus *Aorchis* and the name should be suppressed.

Supplementing the work of MacCallum, I wish to make certain additions to the description of *Heronimus chelydrae*. In the examination of turtles, the heaviest infection found was six flukes in one host, and though the parasite is not uncommon, the relative infection was slight. On the findings in three male and two female turtles, Barker and Parsons endeavor to show the females more heavily infected than the males. Such a conclusion seems unfounded, and in the examination of over fifty infected turtles, I find practically no difference as far as sex of host is concerned.

The body of the living worm is usually curved and often assumes the form of a double bend like an elongated S, the short anterior region becomes concave ventrally and the long posterior part concave dorsally. Movement is slow and sluggish. The size and shape of the parasite have been described by both MacCallum and Barker and Parsons. Apparently the measurements of both were made from fixed specimens, and this can account for the slight difference in their reports. I have observed specimens that measured 18 mm. when fully extended that

did not exceed 12 mm. in the normal characteristic form. The relative width naturally varies with the amount of elongation. MacCallum described the cuticula as sometimes thrown into slight folds or rugae independent of the musculature. Barker and Parsons described strong circular bands of muscle fibers which run around the body at regular intervals and give it a segmented appearance when contracted. I have observed the same conditions, the slight folding of the cuticular covering and also the more pronounced constrictions involving the muscular wall. But in sections I have been unable to demonstrate any regularly occurring bands of strong circular muscle fibers or even an intermittent thickening of the circular muscles of the body wall. The musculature is very weak and slight, no one of the three layers which form the body wall is strongly developed. Lying inside the body wall there is a compact layer of parenchyma (Figs. 3, 5, 6, 9), and this layer increases in thickness anterior to the ovary. Within the outer thickened stratum the parenchyma has the loose vacuolated appearance (Figs. 3, 9) well described by MacCallum.

The measurements of the oral sucker, pharynx, esophagus and intestinal ceca agree with those given by Barker and Parsons, and my examination confirms the histological description of these structures as given by MacCallum.

MacCallum described the ovary as located on the left side of the body. Barker and Parsons' statement concerning the size and position of the ovary agrees with that of MacCallum except that they found the ovary on either the right or left side. My observations confirm the statement of Barker and Parsons; the ovary may be on either the left or right side, but is always on the side opposite from the anterior pigmented region of the uterus. The description of the structures which comprise the oötype as given by MacCallum is entirely confirmed by my observation. A seminal receptacle is present (Fig. 3). It is about one fourth the size of the ovary, situated on the postero-median side of the ovary. It agrees in size and position with Barker and Parsons' description of the testis. In the specimens in which I have been able to trace the course of the uterus, it passes from the oötype to the side of the body opposite from the ovary and posteriad in many coils around the intestine and testis to the posterior end of the body. Then it bends forward and continues anteriad on the ovarian side in similar coils and loops to the level of the ovary, where it turns posteriad and passes diagonally to the opposite side of the body. Here it turns anteriad and soon becomes heavily pigmented. It extends in many loops and folds to or slightly farther than the level of the ovary and then turns caudad, extending as a straight tube in the dorso-median line almost to the posterior end of the body. The pigmentation diminishes as the tube passes caudad and in many specimens disappears about

midway between the ovary and the posterior end of the body. At its posterior end, the descending median section of the uterus turns ventrad and opens into the large, median, ventral sac-like portion (Fig. 7) which extends cephalad to the metraterm. The histological character of the various regions of the uterus has been described by MacCallum and the character of the copulatory organs and position of the genital pore by both MacCallum and Barker and Parsons.

The vitellaria (Figs. 7, 10, 11) consist of two glandular structures which meet anteriorly to form the vitelline receptacle and extend almost to the posterior end of the body. They lie median and ventral to the ceca, the anterior part is tubular, but the central and posterior regions are solid and rodlike.

The eggs are very thin-shelled and, when massed together in the uterus, lose their characteristic shape and become many-sided. Often the shells are lost entirely, the embryos develop eye spots, and in the large sac-like terminal portion of the uterus, there are fully developed ciliated miracidia. This species offers, then, an opportunity to follow the development of the embryo from the maturation and fertilization of the egg to the first larval stage.

The testis (Figs. 7, 8, 10, 11) is a large U- or V-shaped structure; the closed portion is cephalad and situated one fourth to one fifth of the body length from the anterior end. The crura extend caudad to a level about one eighth of the body length from the posterior end. As described by Ward, they are elongate, tubular, irregularly lobed. Their histological appearance is well described by MacCallum and is shown in Figs. 7, 8, 11. Barker and Parsons described a condition in which the testis is atrophied and degenerate, and stated that this is true particularly in the "older larger worms." I have observed the same condition in many individuals of *H. chelydrae*. Often, however, the organ is degenerate and the crura of the testis have shrunk to mere strands of tissue, and sections show the cells in a state of degeneration and disintegration. On the other hand, however, in many of the largest individuals the testis is full-sized and sections show vigorous functional activity of the cells. I am at a loss to account for the atrophy of the testis, but since it occurs in a large percentage of small individuals, and does not occur in many of the largest, I am not inclined to regard the conclusion of Barker and Parsons as satisfactory.

A short vas deferens arises from the anterior part of the testis, turns ventrad and caudad where it opens into the posterior end of the seminal vesicle. No prostate gland was found. The seminal vesicle extends caudad only a short distance from the median part of the testis and the posterior part of the vesicle is usually irregularly coiled. The vesicle extends anteriorly in the ventral part of the body and just anterior to the ovary passes over into the cirrus sac (Fig. 4). This sac is

approximately the same width as the vesicle and the wall is not strongly muscular. The copulatory structures have been discussed.

The excretory system consists of a large, median, dorsal collecting vesicle and smaller ducts distributed throughout the tissue of the body. The large dorsal collecting vesicle extends from the pharyngeal region almost to the posterior end of the body and opens to the exterior in the median dorsal line just posterior to the pharynx. Its walls are often folded and it lies in loose vacuolated parenchymatous tissue. No definite branches from this vesicle were demonstrated. The two largest excretory ducts (Fig. 6) arise in the region of the oral sucker and pass caudad, one on either side of the body, ventral and median to the ceca. They become smaller and more dorsal in position as they extend posteriorly and near the posterior end of the seminal vesicle they disappear in the loose parenchyma.

#### SUMMARY

The present study demonstrates the specific identity of *Heronimus chelydrae* MacCallum and *Aorchis extensus* Barker and Parsons. It confirms the work of MacCallum and includes many additions to the description of the species. The course of the uterus is traced and the position, extent and atrophy of the testis is demonstrated. The wide distribution of the species, and its infestation of six different species of turtles, are items of interest and importance.

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## EXPLANATION OF FIGURES

(All camera lucida tracings)

1. Type specimen of *Heronimus chelydrae*, dorsal view,  $\times 8$ .
2. Ovary and oötype region, from large mounted specimen,  $\times 25$ .
3. Sagittal section showing ovary, seminal receptacle, vitelline receptacle and beginning of uterus,  $\times 57$ .
4. Sagittal section showing excretory pore, genital pore, extent of cirrus sac, and relation of pharynx and esophagus,  $\times 57$ .
5. Cross section at posterior end of pharynx, showing nerve commissure and relation of uterus and cirrus sac,  $\times 57$ .
6. Cross section at level of excretory pore, showing uterus, cirrus sac and small anterior excretory ducts,  $\times 57$ .
7. Cross section near the posterior end of the body, just anterior to the point where the median descending section of the uterus opens into the large ventral sac-like portion. The vitellaria and crura of the testis extend posterior to this level and appear in the section,  $\times 57$ .
8. Sagittal section, lateral to intestine, showing testis and uterine coils,  $\times 27$ .
9. Cross section thru body at anterior tip of ovary,  $\times 48$ .
10. Cross section at the level of the oötype, showing anterior tip testis, tubular nature of the anterior part of the vitelline gland, large sac-like nature of the terminal portion of the uterus and the smaller pigmented descending section of the uterus dorsal to it,  $\times 42$ .
11. Cross section just anterior to the bifurcation of the testis, showing the rod-like character of the vitellaria,  $\times 29$ .
12. Sagittal section showing oral sucker and pharynx, genital pore, uterus and cirrus sac,  $\times 87$ .

## ABBREVIATIONS USED IN FIGURES

<i>cs</i> — cirrus sac	<i>os</i> — oral sucker
<i>cd</i> — excretory duct	<i>ph</i> — pharynx
<i>cv</i> — excretory vesicle	<i>sv</i> — seminal vesicle
<i>cp</i> — excretory pore	<i>sr</i> — seminal receptacle
<i>i</i> — intestine	<i>t</i> — testis
<i>mg</i> — Mehlis' gland	<i>u</i> — uterus
<i>nc</i> — nerve commissure	<i>v</i> — vitellaria
<i>o</i> — ovary	<i>vr</i> — vitelline receptacle
<i>od</i> — oviduct	

STUNKARD—IDENTITY OF HERONIMUS AND AORCHIS

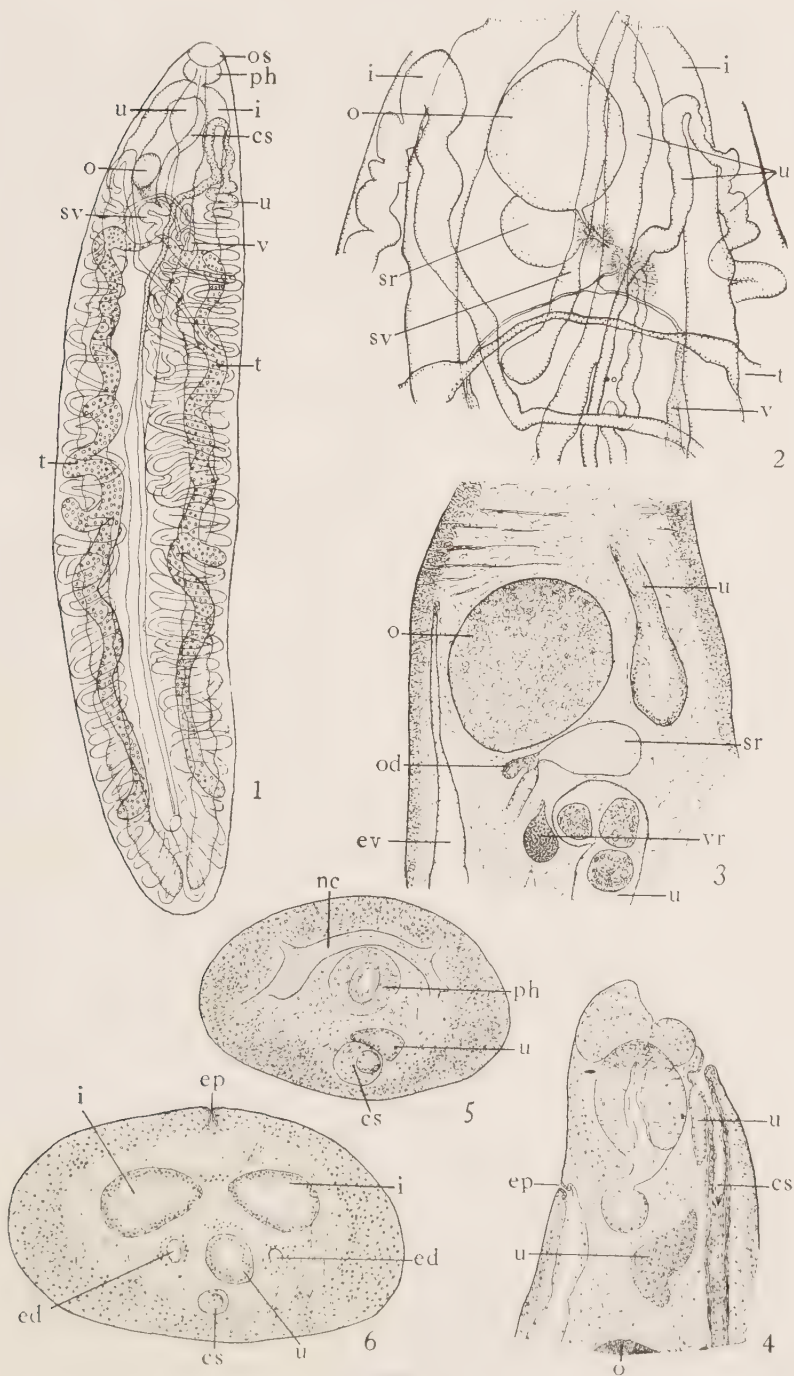


PLATE II



STUNKARD—IDENTITY OF HERONIMUS AND AORCHIS

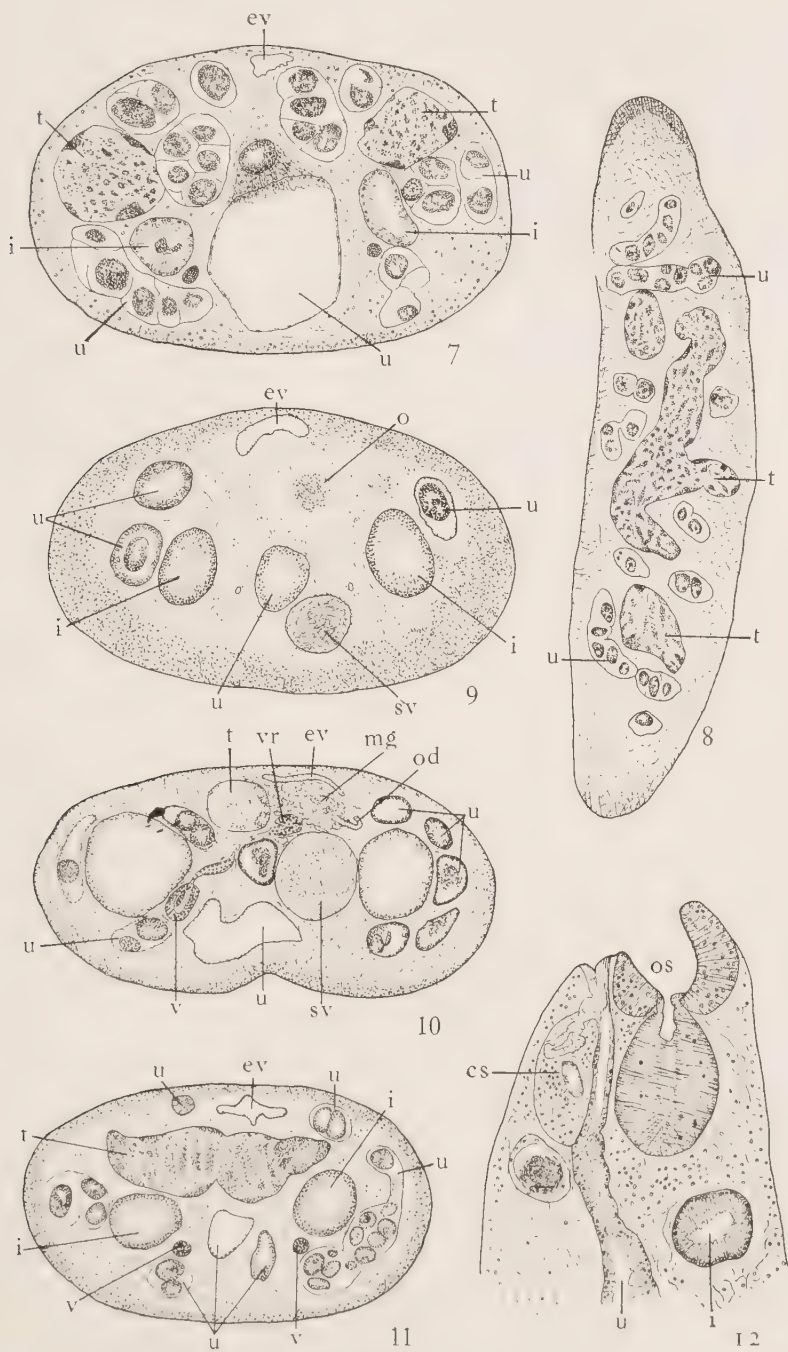


PLATE III



# ON THE MIGRATING COURSE OF ASCARID LARVAE IN THE BODY OF THE HOST

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In my previous paper (Yoshida, 1919) I have reported that the larvæ hatched out in the intestine of a feeding animal, migrate into the liver, lungs and trachea successively and they again pass down the alimentary canal to the intestine, being finally evacuated with the feces sooner or later. The exact course of migration, however, remained to be reported. Since April of this year I have devoted myself to decide experimentally the general course of migration by the larvae in the body of the host. For this purpose, two sets of experiments have been made, (1) experiments injecting larvae into the body of the animal and (2) feeding experiments with the ripe eggs. In the first set of experiments, the migratory power of the larvae was examined chiefly and in the second set the natural and actual course of migration was observed. Both sets of experiments gave successful results from which I have learned the general course of migration from the intestine to the lung.

Before proceeding to describe the experiments, it seems necessary to mention here briefly my methods for injecting larvae and finding them in various organs and tissues. The larvae in the pleural or abdominal cavity are easily collected by centrifuging the fluid in the cavity. As washing fluid 10 or 20 c.c. of physiological salt solution was usually used.

For collecting larvae in any organ, the tissue, such as liver, lungs, etc., is gently brayed in the earthenware mortar with a small quantity of physiological salt solution, the particles of the organ are mixed with 10 or 20 c.c. of physiological salt solution and then filtered through a wire net or gauze of the definite size of mesh to remove the coarse particles. The larvae finally are easily collected by centrifuging the filtered fluid. The coarse particles are to be repeatedly brayed and filtered as before until larvae are no longer present. The larvae thus collected from any organ or tissue are injected with 2 or 5 c.c. of physiological salt solution. Injection was made into the subcutaneous layer, abdominal or pleural cavity of guinea-pig.

## *Injection Experiments*

As to the migrating power of the ascarid larvae, results of two injecting experiments were reported in my previous paper. Since then

numerous experiments were tried by injecting the larvae into the abdominal or pleural cavity, or into the subcutaneous layer of the animal.

EXP. 1.—At 1 p. m., on June 6, larvae collected from the liver of a guinea-pig, killed seventy-five hours after feeding with ripe eggs, were injected into the abdominal cavity of a guinea-pig A. On the next morning after, about twenty hours, the animal was found dead. In the pleural cavity were found two living larvae, but none in the lungs. In the abdominal cavity numerous dead larvae were present and a few in the liver.

EXP. 2.—At 1 p. m., on June 6, larvae from the liver of the same animal as in the preceding experiment were injected into the pleural cavity of two guinea-pigs, C and D. On the next morning the animal C was found dead after twenty hours; in the pleural cavity numerous dead larvae were present and a few living ones in the lungs.

EXP. 3.—The animal D was also found dead in the same morning, the result of examination was the same as in the preceding case.

EXP. 4.—At 8 a. m., on June 4, larvae from the lungs of a guinea-pig, killed sixty-five hours after feeding, were injected into the abdominal cavity of a guinea-pig B. On the next morning the animal was found dead after twenty-four hours. The lungs and liver were infected, many dead larvae being in the abdominal cavity and a few living in the pleural.

EXP. 5.—At 9 a. m., on June 10; larvae from the liver of an animal, killed sixty-six hours after feeding, were injected into the abdominal cavity of a guinea-pig B. The animal was found dead on the next morning after twenty-four hours. Numerous dead and living larvae were found in the abdominal cavity, a few in the liver, five in a piece of the lung and none in the pleural cavity.

EXP. 6.—At 2 p. m., on June 10, the larvae from the liver of the guinea-pig, killed seventy-one hours after feeding, were injected into the abdominal cavity of two guinea-pigs C and D. In the evening of the next day, the animal C was found dead after about twenty-seven hours. On dissection it was discovered that large quantities of the injected matter were accumulated between the skin and the underlying muscle layer. (It was generally observed that the injected particles of the lung or liver with the larvae were gathered into masses in the abdominal or pleural cavity into which they were injected.) In the masses of the injected particles of the liver, larvae were present and dead larvae were found in the abdominal cavity, a few living ones in the liver; they were relatively numerous in the lungs while none were in the pleural cavity.

EXP. 7.—Animal D of the preceding experiment died in the same evening; the result of examination was quite the same as C.

EXP. 8.—A 8 a. m., on June 3, larvae from the liver of a guinea-pig, killed forty-eight hours after feeding, were injected into the abdominal cavity of two guinea-pigs C and D. Animal C was killed at 8 a. m. on the 5th, after forty-one hours. An abundance of larvae were present in the lungs and a few in the liver, while none were found in the pleural and abdominal cavity.

EXP. 9.—Animal D was killed at 3 p. m. on the 5th, after forty-three hours. Numerous larvae were found in the lungs, but none in the pleural cavity and a few in the liver.

EXP. 10.—At 9 a. m., on June 10, larvae from the lungs of a guinea-pig, killed sixty-six hours after feeding, were injected into the abdominal cavity of guinea-pig A. The animal was killed at 4 p. m. on the 12th, after fifty-five hours. A few larvae were present in the lungs and the other organs were unexamined.

EXP. 11.—At 2 p. m., on June 4, larvae from the liver of a guinea-pig, killed after seventy-one hours after feeding, were injected into the abdominal cavity. The animal was killed at 8 a. m. on the 7th, after sixty-six hours. I found two specimens of larvae in the pleural cavity, four in the abdominal cavity, a few in the lungs and a very few in the liver.

EXP. 12.—At 2 p. m., on the 4th, larvae from the lungs of the same guinea-pig were injected into the abdominal cavity of animal B. It was killed at 10 a. m. on the 10th, after ninety-two hours. On the dissection it was found that the injected materials were all introduced into between the skin and the underlying muscle layer. In the accumulated masses of the injected materials larvae were present and a few larvae in the liver as well as in the abdominal cavity. None in the lungs and pleural cavity.

From the observation in this experiment we may easily believe that the larvae migrate into the body cavity through the muscular wall of abdomen.

EXP. 13.—At 11 a. m., on May 10, larvae from the lungs of the guinea-pig, killed after ninety-one hours from feeding, were injected into the abdominal cavity. The animal was killed at 8 a. m., on the 15th, after 117 hours. A few larvae were found in the lungs, but none in the liver.

EXP. 14.—At 11 a. m., on May 11, larvae from the lungs of the guinea-pig, killed after ninety-one hours from feeding, were injected into the abdominal cavity. It was killed at 9 a. m. on the 16th, after 118 hours. The lungs were tolerably hemorrhaged and the larvae were present.

EXP. 15.—At 10 a. m., on May 4, the larvae from the liver of a guinea-pig killed after sixty-nine hours from feeding, were smeared over the abdominal skin from which the hair was removed by cutting closely and shaving. The animal was fixed on the holder during about four hours after operation, restricting the violent movement in order to prevent the loss of larvae and to avoid larvae being taken into the mouth of the animal. After 5 p. m. the animal was put in the separate cage and the smeared part of abdomen was closely covered by the cloth. In the next morning the animal was put in the same cage as others. At noon on the 11th larvae from the liver of a guinea-pig killed after 118 hours, were smeared on the nape of the neck. The animal was killed at 9 a. m. on the 17th, after thirteen and six days from the first and the second smearing, respectively. Five specimens of larvae were obtained from a piece of the lung, two in a part of the large intestine and none in the liver. Almost all these larvae were fully developed, being about 1.8 mm. long. This experiment shows evidently that larvae on the skin may pierce through the body wall of host. It also proves how important and necessary the lungs are for the further development of the ascarid larvae.

From observations in above experiments it may be easily recognized that the larvae injected into the pleural cavity penetrate into the lungs directly, as Experiments 2 and 3 show, and those injected into the abdominal cavity penetrate into the liver or pierce the diaphragm to enter the pleural cavity and thence to reach the lungs. These facts show great power of the larvae in boring through various tissues, as skin, muscle, tendon and several parenchymatous tissues.

Some larvae in the abdominal cavity might have reached the lungs passing through the liver and heart by the way of a blood vessel, but the majority of them are considered to have proceeded directly to the lungs piercing through the diaphragm. It is reasonable to think so from the nature of the larvae, which have marked power in boring

TABLE 1

No.	Date Injected	Injecting Place	Age of Larvae	Date Killed	Duration of Infestation	Pleural Cavity	Lungs	Abdominal Cavity	Liver
1	1 p. m. June 6 A	Abdominal cavity	75 hours from liver	Died morning June 7	< 20 hr.	3	None	Many dead larvae	Present
2	1 p. m. June 6 C	Pleural cavity	75 hours from liver	Died morning June 7	< 20 hr.	Present	A few		
3	1 p. m. June 6 D	Pleural cavity	75 hours from liver	Died morning June 7	< 20 hr.	Present	A few		
4	8 a. m. June 4 B	Abdominal cavity	65 hours from lung	Died morning June 5.	< 24 hr.	A few	Present	Dead larvae	Present
5	9 a. m. June 10 B	Abdominal cavity	66 hours from liver	Died morning June 11	< 24 hr.	None	5	Present	A few (? in a piece)
6	2 p. m. June 10 C	Abdominal cavity	71 hours from liver	Died evening June 11	< 27 hr.	None	Many	Many	A few
7	2 p. m. June 10 D	Abdominal cavity	71 hours from liver	Died evening June 11	< 27 hr.	None	Many	Many	A few
8	3 p. m. June 3 C	Abdominal cavity	48 hours from liver	Killed 8 a. m. June 5	41 hr.	A few	Many	None	A few
9	3 p. m. June 3 D	Abdominal cavity	48 hours from liver	Killed 3 p. m. June 5	48 hr.	None	Many	A few	A few
10	9 a. m. June 10 A	Abdominal cavity	66 hours from liver	Killed 4 p. m. June 12	55 hr.	None	A few	None	None
11	2 p. m. June 4 C	Abdominal cavity	71 hours from liver	Killed 8 a. m. June 7	66 hr.	2	A few	4	A few
12	2 p. m. June 4 D	Subcutaneous	71 hours from lung	Killed 10 a. m. June 8	92 hr.	None	None	A few	A few
13	11 a. m. May 10	Abdominal cavity	91 hours from lung	Killed 8 a. m. May 15	117 hr.	.....	A few	.....	None
14	11 a. m. May 11	Abdominal cavity	91 hours from lung	Killed 9 a. m. May 16	118 hr.	.....	Present		
15	1 p. m. May 4, noon May 11	Smear on abdominal and nape	69 hours and 91 hours	Killed 9 a. m. May 17	13 or 6 days	.....	5 and 2 in large intestine		

tissue. Moreover, the appearance of larvae in the pleural cavity clearly proves their direct migration from the abdominal cavity.

#### FEEDING EXPERIMENTS

Such power of larvae in boring through the tissues makes it reasonable to think that larvae hatched in the intestine may pierce through the intestinal wall to enter the abdominal cavity. This is also surely and actually confirmed by the following feeding experiments.

Feeding experiments were chiefly intended to examine whether the larvae just escaped from the eggshell in the intestine of host may pierce through the intestinal wall or not, and to trace the subsequent course of migration made by the larvae.

EXP. 16.—At noon on June 14, two guinea-pigs A and B were fed with the ripe eggs of sixty-one days old, and the A was killed at 8 a. m. on the next day after twenty hours. The lungs were slightly hemorrhaged, spotted with bloody color here and there, contained numerous larvae of *Ascaris*. Five specimens of larvae were obtained from the pleural cavity, most of them were surrounded by a group or mass of white blood corpuscles and histiocytes. Many larvae were present in the liver as well as in the abdominal cavity, and also three in the spleen, four in the pancreas and two in the left kidney. All larvae found in these organs were quite as young as those just hatched out in the intestine, in size and in organization of body, measuring from 0.2 to 0.24 mm. in length. Thus the larvae found in the abdominal cavity and in other organs may surely be considered to have pierced through the intestinal wall. This was repeatedly proved by the following experiments.

EXP. 17.—The animal B of the preceding experiment was killed at noon on the 15th, after twenty-four hours. The lungs and liver were infected, three larvae in the pleural cavity, two in the spleen, one in the right kidney, three in the pancreas and many in the abdominal cavity. Larvae were all in the same size as in the preceding case.

EXP. 18.—At 3 p. m., on June 12, two guinea-pigs A and B were fed with ripe eggs sixty-four days old. A was killed at 8 a. m. on the 14th, after forty-one hours. The lungs and liver were heavily infected. Five specimens of larvae in the pleural cavity, many in the abdominal cavity, four in the kidneys, one in the pancreas and none in the spleen.

EXP. 19.—Animal B of Experiment 18 was killed at 10 a. m., on the 14th, after forty-three hours. Result of examination was the same as in the case of A, but two larvae were in the pancreas and three in the kidneys.

EXP. 20.—At 3 p. m., on June 1, three guinea-pigs were fed with the ripe eggs of fifty-two (to A and B) and of fifty-three (to C) days old. C was killed at 3 p. m. on the 3rd, after forty-eight hours. The lungs and liver were infected. Larvae were found in the spleen, in the pleural and abdominal cavity. The pancreas and kidneys were unexamined.

EXP. 21.—Animal B of preceding experiment was killed at 8 a. m. on the 4th, after sixty-five hours. The lungs and liver were infected, twelve larvae being in the abdominal cavity, two in the kidneys, four in the pancreas, while none were in the spleen or in the pleural cavity.

EXP. 22.—At 3 p. m., on June 7, two guinea-pigs (A and B) were fed with the mature eggs, fifty-nine days old; B was killed at 9 a. m. on the 10th, after sixty-six hours. The lungs and liver were infected, the former organ being heavily hemorrhaged. Other organs were unexamined, but the spleen with that of A contained nine specimens of larvae.

EXP. 23.—Animal A of Exp. 22 was killed at 2 p. m. on that day, after seventy-one hours. The lungs and liver were in quite the same state of infection as in the preceding case. Eleven larvae were obtained from the pleural cavity, six of them were in the state of free movement, and the remaining five surrounded by the masses of white blood corpuscles and histiocytes. Eight were in the abdominal cavity, four in the kidneys and two in the pancreas.

EXP. 24.—Animal A of Exp. 20 was killed at 2 a. m. on the 4th, after seventy-one hours. The lungs and liver were heavily infected, two specimens of larvae were present in the pleural cavity, one in the spleen, three in the kidneys and many in the abdominal cavity, but none in the pancreas.

Exp. 25.—At 10 a. m. on June 3, two guinea-pigs A and B were fed with ripe eggs fifty-five days old, they were killed at 1 p. m. on the 6th, after seventy-five hours. In A the lungs and liver were infected, four larvae in the pleural cavity, eight in the abdominal cavity, two in the spleen, one in the pancreas and none in the kidneys. In B the lungs and liver were in the same state as in the case A, a few larvae in the abdominal cavity, two in the kidney, but none in the pleural cavity, spleen and pancreas.

TABLE 2

No.	Feeding Date	Age of Eggs, Days	Killing Date	Hours Passed	Pleural Cavity	Lungs	Abdom. Cavity	Liver	Spleen	Pancreas	Kidney
1	Noon June 14 A	61	8 a. m. June 15	20	5	Bloody spot, many larvae	Many	Many	3	4	2 (left)
2	Noon June 14 B	61	Noon June 15	24	3	Slightly blooded present	Many	Present	2	3	1 (rt.)
3	3 p. m. June 12 A	64	8 a. m. June 14	41	5	Many	Many	Many	None	1	4
4	3 p. m. June 12 B	64	10 a. m. June 14	43	Present	Many	Many	Many	None	2	3
5	3 p. m. June 1 C	53	3 p. m. June 4	48	Present	Present	Present	Present	Present		
6	3 p. m. June 1 B	52	8 a. m. June 4	65	None	Present	12	Present	None	4	2
7	3 p. m. June 7 B	59	9 a. m. June 10	66	.....	Heavily blooded present	.....	Present			
8	3 p. m. June 7 A	59	2 p. m. June 10	71	11	Heavily blooded present	8	Present	9	2	4
9	3 p. m. June 1 A	52	2 p. m. June 4	71	2	Heavily blooded present	Many	Present	1	None	3
10	10 a. m. June 3 A	55	1 p. m. June 6	75	4	Heavily blooded present	8	Present	2	1	None
11	10 a. m. June 3 B	55	1 p. m. June 6	75	None	Heavily blooded present	Present	Present	None	2	None

Results of these feeding experiments give the facts accurately in the course of the migration carried out by the ascarid larvae in the body of host, as was surely decided in combination with the result of above injecting experiments.

Larvae hatched out in the intestine immediately proceed to the abdominal cavity piercing through the intestinal wall. Larvae in the cavity may wander everywhere freely, that is, some to the liver, spleen, pancreas or kidneys in the cavity and others piercing the diaphragm to enter the pleural cavity, finally penetrating into the lung from its surface. Thus it is believed that the larvae migrate in every direction,

boring through various organs or tissues by means of their own power of piercing, but not by the way of blood vessels.

As to the course of larvae migrating from the intestine to the lung F. H. Stewart supposed two ways and said: "1. Boring through the wall of the stomach or intestine the larva enters a mesenteric venule and is carried to the liver. Thence they pass in the hepatic vein to the heart and by the pulmonary artery to the lung. 2. The larva after hatching in the stomach or duodenum travels by the bile duct and through the degenerated liver tissues and reaching a hepatic venule continues its course as in the first case."

Stewart's supposition might be true, but it would be an accidental case and not the sole or general course of migration, I think. If a blood vessel is the only way by which the larvae migrate in the body of host, the appearance of larvae in the pleural cavity is very difficult to explain.

It was formerly considered by Stewart as well as myself that larvae migrate successively from the intestine to the lung, passing through the liver, in consequence of which the larvae appear in the lungs later than in the liver. From this belief it was easy to think the larvae travel in the blood vessel from the liver to the lung, passing through the heart. But the belief in this successive migration of larvae has been radically destroyed by my above experiments.

The larvae in the abdominal cavity may easily wander about everywhere, and penetrate not only into various organs or tissues in the cavity, but into the lungs, piercing through the diaphragm. Thus the liver, with several other organs in the abdominal cavity, is not a necessary and important organ to be passed for the larvae to reach the lung, as we formerly considered. Larvae penetrating into the liver might be considered to travel to the lungs by the way of a blood vessel, but it is not the general way for larvae to reach the organ. Hence I am inclined to believe that the larvae migrate from the intestine to the lung by their strong power of boring through the various organs or tissues, but not by the way of a blood vessel.

The general and important course of migration by the ascarid larvae in the body of host may be as follows: The ascarid larvae escape from the eggshell in the intestine of host and proceed to the abdominal cavity by boring through the wall of intestine. Thence they pierce the diaphragm to enter the pleural cavity, finally penetrating into the lungs from their surface. It might be considered as an additional and mere accidental course of migration that the larvae in the abdominal cavity penetrate into the liver, thence they are carried to the lungs by the way of blood vessels passing through the heart. This belief in the course of migration of the larvae is strengthened by histological study of the infected organs and tissues. It shows that almost all the larvae in the

lungs and liver are not found in the blood vessels but in other tissues. Details on this side, however, will be reported in another paper.

Furthermore, the lungs are the only necessary and important organ to be passed by the larvae in the course of their development. In this organ the larvae stay longer than in any other, and they develop rapidly there as completely as possible, reaching a length of about 1.7 to 2.0 mm. Larvae in the lungs, several days after feeding, are generally much larger than those in any other organ. This fact was repeatedly observed in recent experiments. The results of some experiments on this subject are given in Table 3.

TABLE 3

Duration of Infestation	Length of Worm in						
	Lungs	Pleural Cavity	Abdominal Cavity	Liver	Spleen	Pancreas	Kidney
162 hrs.	0.3-0.57 0.95-1.0	0.28 0.48	0.28 0.48	0.28 0.48			
166 hrs.	0.4-0.51 0.85-1.33	0.28 0.46	....	0.4	0.28	0.28 0.44	0.28
250 hrs.	1.8 2.0	0.3	0.28				

The larvae in the lungs continue their development and migrate to the mouth cavity through the trachea, again passing down the alimentary canal to the intestine of the host.

The occurrence of larvae in the spleen was first mistakenly reported by Stewart in his first paper, and corrected in his second paper. Afterward B. H. Ransom and W. D. Foster recognized the presence of larvae in the organ. I have frequently found the larvae not only in the spleen but in the pancreas and kidneys. The occurrence of larvae in these organs is easily explained by their power in boring through tissues. It should be, therefore, clear that the larvae might penetrate into other organs or tissues such as ovarium, adrenal body, etc., which as yet I have not examined. All these organs and tissues are accidentally or temporarily visited by the larvae and in them they are not able to complete or continue development. Larvae in the liver, however, might be carried in the hepatic vein to the heart and consequently by the pulmonary artery to the lungs, where they can develop further. The fate of larvae in organs or tissues other than the lung is not yet traced accurately, experiments on this problem being under way. From the results of some experiments, however, I have learned the following facts: After several days (five to ten) after feeding, few larvae or none were found in these organs, but they were more or less present in the abdominal and pleural cavity. The more time passed the more the larvae decreased in number in these organs while the more they increased in the lung. The larvae in these organs were

much smaller than those in the lung, and some of the former were dead or decomposed in structure. So great variation is found in size of larvae in the lung that it cannot be caused by the individual difference only. The larger specimens were three or four times as large as the smaller one, which was in the same size as those in the liver and other organs or tissues. This great variation is probably due to a different invasion of larvae. It is easy to see that the rapid growth of larvae in the lungs may cause great difference in size according to difference in time of invasion.

From these facts we may infer that some larvae in these organs may go back in the abdominal cavity and proceed to the pleural cavity to invade the lungs, the important place for their further development, and those remaining probably perish in these organs. At any rate, the details of larval fate in these organs, except the lung, should be left untouched here.

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ON THE LIFE HISTORY OF *DAVAINEA TETRAGONA*  
(MOLIN), A FOWL TAPEWORM \*

JAMES E. ACKERT

Further experimental studies by the writer on the life histories of fowl cestodes have demonstrated that tapeworms which appear to be *Davainea tetragona* (Molin) may be transmitted to *Gallus domesticus* by feeding them *Musca domestica* Lin. That this fly may be the means of transmitting other fowl cestodes is known. Grassi and Rovelli (1892: 33, 87) found in its body larvae whose scolices closely resembled those of *Choanotaenia infundibuliformis* (Goeze), while Gutberlet (1916: 235) succeeded both in infecting *M. domestica* with cysticerchi by feeding onchospheres of this tapeworm, and in rearing adult worms by giving to fowls house flies taken from nature. And the writer (1918: 41) reared *Davainea cesticillus* (Molin) in *Gallus domesticus* by feeding *M. domestica* which some weeks previously had been given onchospheres of this cestode.

In the present experiment, chicks hatched in incubators were taken under cover to one of two experimental feeding houses which had been fumigated ten hours with sodium cyanide\* and thoroughly cleaned. The cement floors and 18-inch walls excluded worm-like animals, while the screened openings and enclosed vestibules facilitated in eliminating winged forms. On entering the vestibule any intruder was captured before proceeding to the interior of the feeding house. Extreme care was exercised in administering the food, the uncooked portion containing no animal tissues except occasional feedings of fresh beef, and of course, the experimental feedings. By these methods, which have been employed during the last five years, control chicks running with the experimental ones have been free from parasitic worms in every case. It is possible that an occasional arthropod may enter through the fourteen-mesh window screens, but the control chicks have equal opportunity with the experimental ones for ingesting such forms. The chances of such animals being infected with fowl tapeworm larvae are minimized owing to the fact that the experimental feeding houses are 250 yards from a poultry yard.

In September, 1918, several poultry yards in the vicinity of Manhattan, Kansas, were visited. Spring chicks were examined, and at two places *D. tetragona* were among the tapeworms found. In the

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chicken houses and under roosts the freshly voided feces were covered with flies, many of which were *M. domestica*. At these places large fly traps were set near the chicken houses and thousands of flies trapped. From day to day the traps were collected and immersed in tap water for several hours to facilitate identification of the flies. It was found that the latter could be removed from the traps, slightly dried, and the *M. domestica* sorted out one by one before they recuperated sufficiently to escape. They were then given to chicks reared in the experimental feeding houses. When the flies were immersed seventeen hours and then placed by an open window, large numbers recovered fully in two and one-half to three hours after their removal from the water. Among the lots of *M. domestica* given to the experimental chickens were many flies making random movements indicating that any possible tapeworm larvae in their bodies were probably uninjured by the immersion.

Numerous *M. domestica*, obtained in this way, were given as follows to chicks 2½ months old during the autumn of 1918: 3,506 flies to each of chicks 233, 234 and 235 in eighteen feedings, ranging from thirty-nine to 493 between September 23 and October 19; 3,467 flies to chick 236 in seventeen feedings, varying from eighty-nine to 493 between September 24 and October 19; 2,938 flies to each of chicks 239, 240, 241, 242, 243, 245, 246 and 247 in fifteen lots of flies, numbering from 260 to 493 between September 30 and October 19, and 2,026 flies to each of chicks 248, 249, 250 and 251 in thirteen feedings of fifty to 290 from October 4 to October 19.

Early in November, six weeks after the first feeding of flies, four chicks were examined. Two of these were negative, but the other two had mature tapeworms in their intestines, chick 235 having ten long worms, and chick 250 two medium-sized ones, all of which were sexually mature, and some of them possessed gravid proglottids. Two of the control chicks which had been running with the experimental ones were killed, and the examination showed that their intestines were free from parasitic worms. One month later two large tapeworms were found in the intestine of chick 249. During the next eighteen days ten control chicks were examined, and every one of them was free from helminths. In the same period, examinations of the remaining eleven experimental chicks showed that none of these was infected.

Morphological studies of several of the cestodes obtained are here recorded. These tapeworms vary in length from 31 to 307 mm., and from 1 to 3 mm. in width. The scolices measure 247 to 410 $\mu$  long by 187, to 394 $\mu$  wide, and are provided with retractile rostellum varying from 49 to 57 $\mu$  in breadth, each armed with a single row of about 100 hooks, 7 to 8 $\mu$  long. A short dorsal root and a long ventral one

are characteristic of each rostellar hook which is provided with a small, recurved prong. The oval suckers measure 94 to 170 $\mu$  in longitudinal diameter by 53 to 82 $\mu$  in transverse diameter, and are armed with eight to ten rows of minute hooks varying in length from 5 to 8.75 $\mu$ . Of the two short roots, the ventral is slightly the longer, but it is shorter than the prong, which is slender and curved. The neck is long and slender. The proglottids are trapezoidal and imbricate, and the edge of the strobila is serrate. Nearly all of the proglottids are broader than long, the width of the anterior ones being two and one-half to four times the length, that of the middle ones, two to two and one-half times the long axis, and the breadth of the posterior segments, twice the length or about equal to it, depending on the age of the worm. The unilateral genital pores, numbering one in each segment, are situated at or in front of the middle of the lateral margin and are marked occasionally by papillae. The vas deferens and vagina pass on the dorsal side of the nerve, but between the excretory vessels.

Of the male genitalia, the testes, numbering 21 to 25, lie in the median portion, mostly on the aporose side of the proglottid. The vas deferens lies in the anterior third of the segment; it begins near the middle and extends laterally in a much convoluted course to the base of the cirrus pouch which it enters; after two or three coils it becomes continuous with the cirrus which, apparently, is without spines. The pyriform cirrus pouch, measuring 77 to 84 $\mu$  in length, is surrounded by a layer of muscles 3.5 $\mu$  thick.

A branched ovary in the middle of the proglottid is the most conspicuous part of the female genitalia. Posterior and slightly to the left of the ovary is the yolk gland, somewhat reniform, with a diameter ranging from 90 to 114 $\mu$ . The shell gland, lying in front of the yolk gland and dorsal to it, may vary from 52 to 60 $\mu$  in diameter. The vagina begins at the genital pore posterior to the cirrus pouch; at first it is slender, but at a distance of about 15 $\mu$  it widens into a thin-walled tube adapted for a seminal receptacle which extends transversely across the proglottid, passing dorsal to the nerve and between the two excretory vessels to the middle of the segment where it bends posteriorly and ventrally, joining the oviduct immediately behind the ovary. After connecting with the vitelloduct in the shell gland, the oviduct extends forward and ends on the dorsal side of the ovary. A persistent uterus is not formed. The eggs pass from the distal end of the oviduct and become imbedded in a fibrous, granular mass which ultimately fills most of the proglottid. This structure divides into 40 to 114 parts, forming egg masses, each of which contains six or more eggs, and is surrounded by a membrane. The individual egg has three envelopes: an inner, surrounding the onchosphere; a middle, some-

what wrinkled; and a smooth, outer covering. The diameter of the onchosphere is approximately  $11\mu$ , while that of the outer envelope is about  $46\mu$ .

From these studies it seems evident that several of the cestodes obtained are *Davainea tetragona* Blanchard 1891 (in part<sup>1</sup>). Certain variations in structure occur which, hitherto, have not been recorded for this cestode, but they are more nearly in accord with *D. tetragona* than with the somewhat closely related species, *D. echinobothrida* Blanchard 1891. The length of the longest of these cestodes is 307 mm., while the maximum length recorded for either of the above species is 250 mm. Of these tapeworms the maximum width of scolex is  $394\mu$  as compared with  $350\mu$  given by Ransom (1904:278) for this worm, and  $450\mu$  by the same author for *D. echinobothrida*. The maximum longitudinal diameter of the suckers of the cestodes in question is  $170\mu$ , which exceeds the maximum recorded size for this worm  $60\mu$ , and lacks  $30\mu$  of attaining the greatest diameter of the suckers of *D. echinobothrida*, according to Gutberlet (1916:36). In these cestodes, the vagina and vas deferens pass between the excretory vessels instead of dorsal to them as is usually recorded for *D. tetragona*. The number of egg masses reported for this tapeworm varies from 50 to 100, but gravid proglottids of the cestodes under consideration may have from 40 to 114 such masses. These small differences in size and structure, in the writer's opinion, should be considered only as variations in the morphology of *D. tetragona*.

The evidence presented points to *M. domestica* as an intermediate host of *D. tetragona*, but thus far larvae of this tapeworm have not been found in the house fly. As the onchospheres are in masses several times the diameter of an embryo, it seemed possible that *M. domestica* might not be able to ingest them. Accordingly, tests were made. Lot 424, consisting of six *M. domestica* were offered fifty egg masses from a live proglottid in a small drop of sweetened water on a glass slide. In three minutes two of the flies took all the moisture, whereupon the slide was examined microscopically, and of the fifty egg masses twenty-nine remained on the slide. In a second similar trial sixteen egg masses were taken, and in two others, six masses were ingested. Moreover, the walls of some of the egg masses had been ruptured by the sucking of the flies which drew out some of the enclosed oncho-

1. R. Blanchard (Notices Helminthologiques. Mem. Soc. Zool. France, 4:436) records a double row of about 200 rostellar hooks, and circular suckers for this worm. The latter are clearly oval in all of these specimens. Under ordinary magnification the rostellar hooks appear to be arranged in a double row, but with the aid of an oil immersion objective individual hooks may be seen throughout their length, and their number in a definite arc of the rostellum counted.

spheres. The adaptability of flies' feet for carrying small organisms made a careful scrutiny for the adherence of egg masses imperative. In one case a mass was carried approximately an inch before it fell from the foot of the fly. These tests showed that egg masses of *D. tetragona* can be ingested by *M. domestica*, and that the latter may take separate onchospheres from the egg masses.

Having determined that onchospheres of this tapeworm are ingested by *M. domestica*, two questions arose: Are ingested egg masses or onchospheres regurgitated by the flies and lost? Do the onchospheres pass through the digestive tract of the flies unaltered? It is known that *M. domestica* after gorging itself with liquid food usually regurgitates, forcing out of the crop through the proboscis a portion of this food. The latter frequently takes the form of a bubble adhering to the withdrawn proboscis. At other times the regurgitated drop of liquid is placed on some object and, according to Graham-Smith (1913: 69, 86), it is then gradually taken into the stomach of the fly. In such cases the object is marked by a relatively large, regurgitation spot with a characteristic, light center surrounded by an opaque, marginal ring. These flat regurgitation spots are easily distinguished from the cone-shaped fecal specks of smaller diameter and darker color. In regurgitating it would not be surprising if ingested egg masses or separate onchospheres would be left in the regurgitation spots. To determine this point, small test cages were constructed, every part of which was capable of being examined by the compound microscope. The four walls were made of glass slides, 38x75 mm., the corners being sealed with melted paraffin. The top of the cage consisted of a glass plate 38 mm. square, which was held in place by strips of gummed labels, while a glass slide served as the base of the cage. In these test cages, the house flies behave normally and lived several days.

As the answers to both questions were obtained from the same experiments, the preliminary tests for solving the second question are given here. To ascertain whether or not the onchospheres go through the flies unaltered, it was necessary to learn the length of time required for the passage of food through their digestive tracts. Several tests were made, a typical one being described in detail. Three *M. domestica* (lot 309) taken from a trap baited with vinegar and corn syrup, were placed in one of the test cages where they were given a few drops of sweetened water colored red by the addition of carmine grains. That the flies fed freely was evident from the bright red appearance of the anteroventral portions of their abdomens—the areas immediately below their crops. Each fly regurgitated once. After fifteen minutes the base slide with the remaining red liquid was removed and a clean

one substituted. Two hours and thirty-eight minutes later four characteristic red fecal specks appeared. During the ensuing three hours, six specks, respectively, were defecated, while a maximum of ten were deposited during the seventh hour after feeding. In the next two hours the number of specks in the cage was increased by only five. The tenth hour showed an addition of six faintly red fecal specks, and in the eleventh hour after feeding there were four defecations which contained so few carmine grains that the latter were visible only by aid of a microscope.

To be certain that the red color of the fecal specks was due to the carmine and not to the vinegar and syrup with which the trap had been baited, the fecal deposits from lots 283-303 of *M. domestica*, isolated in lantern globes from the same trap, were carefully examined. Not one of the several hundred specks was colored red, and examination with a lens confirmed the absence of any red particles.

Other similar tests for ascertaining the length of time required for food to pass through the digestive tract of *M. domestica* were made with fresh blood. In these cases, the feces were voided in nine to twelve hours which is approximately the same period as that determined by Gutberlet (1916:233) in similar tests on flies. On the other hand, sweetened water and carmine grains, before mentioned, were voided in at least two hours and thirty-eight minutes, and in a test with whole, sweet milk and powdered carmine the bright red fecal specks appeared in one hour and fifty-five minutes. The evidence here presented indicates that the time required for liquid food to pass through the digestive tract of *M. domestica* may vary from one hour and fifty-five minutes to twelve hours.

Having ascertained the approximate period of time required for the passage of liquid food through the body of the house fly, attention was directed to the questions of the egg masses or onchospheres being lost by regurgitation, and of their passing through the alimentary canal unaltered. To determine these points, lots 430 and 431, consisting of six and four *M. domestica*, respectively, were isolated in test cages. Gravid proglottids of *D. tetragona* from freshly voided chicken feces were teased in slightly sweetened tap water. In this medium, fifty-six egg masses were offered to lot 430, and ninety to lot 431. The first lot took seven masses, and the second twelve. As each mass contains six or more onchospheres, lot 430 took at least thirty-six of these embryos and lot 431 a minimum of seventy-two. After feeding the egg masses, each lot of flies was transferred to a clean test cage, whereupon the vacated cages were pried apart and every regurgitation spot examined by the aid of a compound microscope. No structure resembling either an egg mass or an onchosphere

was present in any of the spots. At two-hour intervals the flies were transferred to clean test cages, and each fecal speck studied, a mechanical stage being used to locate the fecal deposits. The examination of these specks was continued until the nineteenth hour after feeding the egg masses. In one instance a globular-shaped structure about the size of an onchosphere occurred in a speck, but it lacked hooks and internal membranes.

From this test and from those made on the eggs of *D. cesticillus* which will be reported in a later paper, it seems evident that few, if any, ingested onchospheres of the cestode in question are either lost by regurgitation or passed through the digestive tracts of these flies unaltered. This evidence and the morphological studies on fourteen adult tapeworms obtained by giving common house flies from infected poultry yards to experimental chicks lead the writer to conclude that *M. domestica* may be the means of transmitting *D. tetragona* from one fowl to another.

#### SUMMARY

1. Flies trapped at poultry yards infested with *Davainea tetragona* (Molin) and other tapeworms were given (many alive) to young chicks reared in a screened house. The food of all controls was free from animal tissues.

2. In two months the chicks were examined; three contained mature tapeworms, several with embryos. The twelve control chicks were free from parasitic worms.

3. Flies eat both onchospheres and egg masses of *D. tetragona*, and neither when ingested are lost by regurgitation, or passed through the digestive tract unaltered.

4. As common house flies from infected poultry yards constituted the only difference between the food given to the experimental chicks and that fed to the control chicks, evidently *D. tetragona* may be transmitted from one fowl to another by *M. domestica*.

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ON THE LIFE HISTORY OF THE CHICKEN CESTODE,  
*HYMENOLEPIS CARIOCA* (MAGALHAES)\*

JOHN E. GUBERLET

The problem of tapeworm infestation in chickens has received some attention during the last few years. The investigations being carried on at present are chiefly those with regard to the life cycle of the various forms. Of the six species found in the United States three have been demonstrated experimentally. *Davainea proglottina* (Davaine) was transmitted experimentally to chickens through the slug, *Limax cinereus* Lister, by Grassi and Rovelli (1889:372; 1892:86). This species has been reported from only a very few localities in this country. *Choanotaenia infundibuliformis* (Goeze) was transmitted through the common house fly, *Musca domestica* Linn., by the writer (Guberlet 1916a:235; 1916b:30). *Davainea cesticillus* (Molin) has also the house fly, *Musca domestica* Linn., as its intermediate host (Ackert 1918:41).

Recently, the writer has demonstrated experimentally that the stable fly, *Stomoxys calcitrans* Linn., may transmit to chickens another tapeworm, *Hymenolepis carioca* (Magalhaes 1898). The chickens used in these experiments were hatched in an incubator and placed as soon as coming from the eggs in insect proof cages. Great care was taken in feeding the birds so that no insects entered the cages or were given to the birds with the food. The chicks were fed grain and a small amount of green feed which was carefully inspected.

The experiments were carried on at two different times and the cestodes were obtained from the birds on both occasions by postmortem examinations. In August, 1914, on a farm at Hardy, Nebraska, the writer placed six chicks as soon as hatched into an insect-proof cage. Three were used in the experiment and the remaining three were used as controls. Large numbers of *Stomoxys calcitrans* were taken on August 18, 19 and 20 from around the chicken house and yards and given to three of the chicks. The chicks were all killed on August 29 and two (one was two and one-half weeks old) of the three experimental chicks each harbored seven small cestodes. The other chick as well as the three controls were free from parasites. The writer was compelled to give up the work at that time on account of a change of location and consequently could not carry on the experiments any farther until the autumn of 1918. On December 16-19 seventy-seven

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\* Contribution from the Parasitology Laboratory of the Oklahoma Agricultural Experiment Station.

flies, *Stomoxys calcitrans*, were given to a chick reared in an insect-proof cage at the Poultry Plant of the Experiment Station at Stillwater, Oklahoma. More flies could not be obtained at this time of the year because of cold weather. This chick was killed on February 11, 1919, and upon examination was found to harbor three mature worms of the species *Hymenolepis carioca*. From the same cage twenty-four other chicks used for other experiments were killed and in no case was there an infection with this species.

At the time when this experiment was carried on in Nebraska the infestation with *Hymenolepis carioca* was very heavy in nearly all of the chickens on the farm and at the same time the *Stomoxys calcitrans* were particularly numerous. At the Poultry Plant of the Oklahoma Experiment Station this species of cestode was not common until in November and December when the chickens became very heavily infested. During this period the *Stomoxys calcitrans* also were very abundant about the poultry yards. This was more evident on account of the scarcity of other species of flies. At this season of the year these flies seem to be somewhat sluggish and inactive and consequently become easy prey for chickens.

Large numbers of flies of the species *Stomoxys calcitrans* were fed on onchospheres and fragments of mature proglottids of *Hymenolepis carioca*. The flies during the course of the feeding experiment were fed on milk, syrup and small amounts of sterile chicken droppings which they ate very readily. The flies were kept alive as long as possible and when they died they were preserved for sectioning.

*Hymenalepis carioca* (Malgalhaes 1898) Ransom 1902

*Diagnosis:* Length 20 to 110 mm. Breadth at neck 75 to 150 $\mu$ , at posterior end 0.4 to 0.8 mm. Segments three to five times or more broader than long throughout strobila. Head (Fig. 1) flattened dorso-ventrally, 140 to 160 $\mu$  long, 150 to 215 $\mu$  wide and 100 to 150 $\mu$  thick. Suckers shallow, 75 to 100 $\mu$  in diameter, armed with hooks (Fig. 2) 6 to 8 $\mu$  in length with short ventral root and dorsal root a mere knob. Rostellum (*r*) unarmed; in retracted position 25 to 45 $\mu$  in diameter and 90 to 110 $\mu$  in length with a small pocket (*rp*) opening to exterior in anterior position. Unsegmented neck portion of strobila 0.6 to 1.5 mm. long. Genital pores almost entirely unilateral, a single pore being located in each segment slightly in front of middle of right hand margin.

*Male Reproductive Organs:* Testicles three in number, normally two on left and one on right of median line. On dorsal side of inner end of cirrus pouch vas deferens is swollen into prominent seminal vesicle (*sv*) which may attain a size of 70 by 50 $\mu$ . Cirrus pouch (*cp*) in sexually mature segments 120 to 175 $\mu$  long by 15 to 18 $\mu$  in diameter; almost cylindrical, slightly curved toward ventral surface of segment;

on outer surface about 20 longitudinal muscle bands, 2 to  $3\mu$  in thickness, very prominent in cross section; vas deferens enlarged within cirrus pouch to form small reservoir occupying proximal portion of pouch; distal portion of vas deferens within pouch very slender and functions as cirrus. Genital cloaca 12 to  $20\mu$  deep.

*Female Reproductive Organs:* Opening of vagina in floor of genital cloaca, ventral and posterior to cirrus opening. First portion of vagina very narrow, 1 to  $2\mu$  in diameter. Vagina passes inward past excretory canals and in sexually mature segments becomes swollen into prominent seminal receptacle (*sr*) which extends forward to anterior border of segment and inward to proximal end of cirrus pouch. Ovary (*o*) faintly bilobed or trilobed in posterior half of proglottid. Yolk gland (*y*) spherical or ovoid 30 to  $40\mu$  in diameter, situated near median line of segment, posterior and dorsal of ovary. Uterus at first a solid cord of cells extending transversely across segment along anterior border of ovary; becomes hollowed out and grows backward on dorsal side of ovary; in gravid segments (Fig. 4) occupies nearly entire segment and filled with eggs. Embryos (Fig. 5) in gravid uterus spherical or oval, with four membranes, the two middle membranes often approximated to form thick layer which shows a somewhat granular structure. Diameter of outer membrane 38 by  $38\mu$  to 80 by  $75\mu$ , of outer middle membrane 32 by  $32\mu$  to 70 by  $65\mu$ , of inner middle membrane 26 by  $26\mu$  to 45 by  $40\mu$ , of inner membrane 24 by  $18\mu$  to 32 by  $24\mu$ . This membrane lies so close to onchosphere that it is almost impossible to distinguish it from embryo. The embryonic hooks penetrate this membrane. The onchosphere is 22 by  $16\mu$  to 30 by  $22\mu$  in diameter; onchospheric hooks (Fig. 6) are 8 to  $10\mu$  in length.

This thread-like worm, according to the above observations, seemed to be most numerous during the late summer and fall at the seasons of the year when *Stomoxys calcitrans* are very abundant. During the autumn this species of fly is less active and consequently is more easily taken by chickens. Experimentally infesting chicks with *Hymenolepis carioca* through feeding infested stable flies *Stomoxys calcitrans* under control conditions makes it evident that this species of fly may be the intermediate host of this species of chicken cestode.

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## EXPLANATION OF PLATE

- Fig. 1.—Scolex of *Hymenolepis carioca*,  $\times 160$ .  
Fig. 2.—Hooks from suckers. Free hand drawing,  $\times$  about 2000.  
Fig. 3.—Reconstruction of proglottids showing organs,  $\times 200$ .  
Fig. 4.—Section of ripe proglottids showing gravid uterus,  $\times 40$ .  
Fig. 5.—Embryos with membrane,  $\times 600$ .  
Fig. 6.—Onchospheric hooks,  $\times 600$ .  
Drawings made with aid of camera lucida.

## ABBREVIATIONS

<i>cp</i> — cirrus pouch	<i>rp</i> — rostellar pocket
<i>dex</i> — dorsal excretory canal	<i>sr</i> — seminal receptacle
<i>vex</i> — ventral excretory canal	<i>sv</i> — seminal vesicle
<i>o</i> — ovary	<i>t</i> — testes
<i>r</i> — rostellum	<i>y</i> — yolk gland

GUBERLET—LIFE HISTORY OF CHICKEN CESTODE

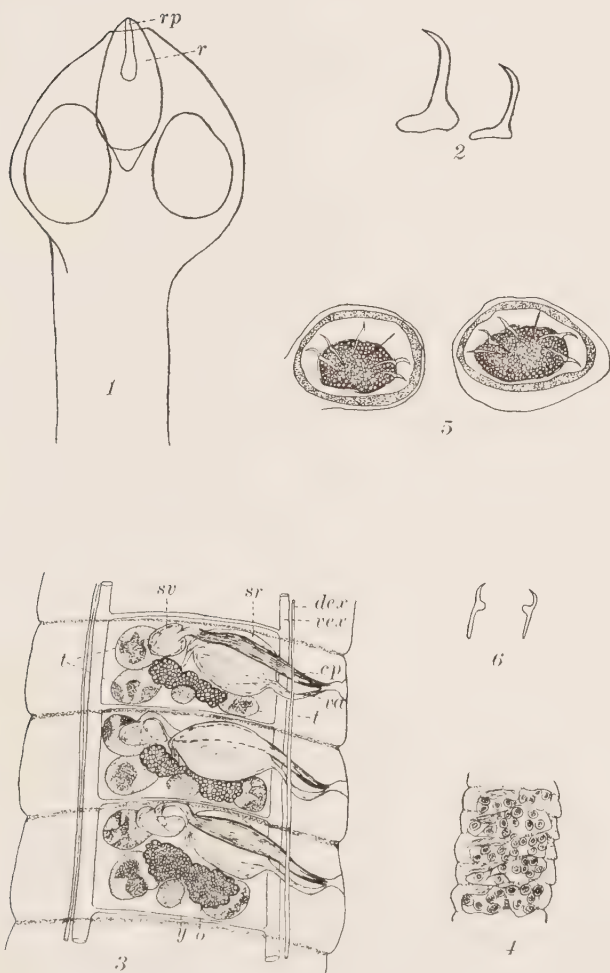


PLATE IV



# FURTHER NOTES ON THE STUDY OF THE HUMAN LUNG DISTOME, *PARAGONIMUS WESTERMANI*

KOAN NAKAGAWA

(From the Government Hospital, Taichu, Formosa)

## I. CERCARIA FOUND IN FRESHWATER SNAIL

Since 1915, when I first discovered seventeen species of cercariae infesting the mollusks found in the rivers in Shinchiku Prefecture, Formosa, I have attempted to infest with the miracidia of the human lung distome the river snails, in which the miracidia of other species can develop into cercariae, and thus to learn the complete life cycle of the parasite in question. I thought that one of the seventeen different forms of the cercariae I examined belonged to that of the human lung distome for the following reasons:

(a) This is the only form found in the aboriginal villages, where human lung distomiasis is most prevalent.

(b) Both the miracidia of the human lung distome and this species of cercaria prefer a particular water snail to others as hosts.

(c) Similarity in shape of the spine in the oral sucker and of the excretory vesicle of both the encysted larvae of the human lung distome in the crab and this species of cercariae.

To prove the above conjecture a great many devices were tried, but all turned out to be futile, due probably to the lack of good tap water. It was very difficult to keep the water-snail alive in the aquarium long enough to finish the infection experiments. Finally a live box was made and immersed in the river. In this box both the water snails having the supposed cercariae of the human lung distome and the crabs free from previous infection were put together. This experiment also failed. But I found in the crabs the youngest encysted larvae that seems to have just entered. They were supposed to be those of the human lung distomes, since similar larvae have been reported from Japan proper—Niigata, Gifu, Okayama and Tokushima prefectures. So I reported elsewhere the encysted larvae I found were those of the human lung distome.

In the spring of 1917, Dr. Yokokawa and myself discovered a new species of the encysted larvae infesting the crabs found in the infected regions of Shinchiku Prefecture in Formosa. They were identified to be those of *Stephanolecithus parvus* n.g., n.sp., that is a species independent of the human lung distome. It may be objected that there may be more than one species of cercariae infesting the water snails found in Japan proper, and mine may not actually be those

of the human lung distome. To meet this objection investigation was resumed in October, 1917, at Kalapai. To my great astonishment, there I could find four different species of cercariae. No. 12\* (80.0 per cent), No. 15 (13.3 per cent.), No. 4 (3.3 per cent.), and the newly discovered one (16.6 per cent.). Besides, I came across two forms of redia, of unknown species. Usually only one species of cercaria is found in the water snails, but there are also many cases in which more than one species of the cercariae are found in one individual. This newly discovered cercaria seemed more closely related with that of the human lung distome than No. 12 does.

The cercaria No. 12 was discovered by myself in January of 1918, widely distributed in the water snails found in the rivers running through the villages free from infection, such as Ako, Tainan, Kagi and Nanto. This makes their identity to the human lung distome extremely doubtful. The newly discovered cercaria will therefore be described in some detail.

This cercaria was discovered in May, 1917, by myself in *Melania libertina* G. found in the rivulet in Torunsho in Shinchiku Province, and in December of the same year, in the same species of the water snail in Kalapai. It is oblong, 0.2 to 0.25 mm. long by 0.08 to 0.1 mm. wide. The oral sucker is large, its diameter being 0.06 mm. It is provided with a sharp spine. The abdominal sucker is smaller than the oral and has the diameter of 0.04 mm. Around the sucker is a group of glandular cells; the glandular ducts run toward the anterior end of the body with a wavy course. The excretory vesicle is a straight tube appearing like a slit and lies on the median line arising very closely to the abdominal sucker and running toward the posterior end of the body. The tail is very small, having the length of 0.02 to 0.03 mm. and the breadth of 0.01 to 0.015 mm. At the posterior end of the tail are several short spines arranged in a row. The cercaria moves fairly lively.

The redia which gives rise to the cercaria was also found. Young rediae are spheroidal, 0.1 to 0.2 mm. in diameter, or spindle shaped, 0.2 mm. long by 0.1 mm. wide. The full grown rediae may either be spindle shaped or cylindrical, with the length of 0.3 to 0.7 mm. by the width of 0.15 to 0.3 mm. They have a pharynx 0.1 mm. in diameter and a voluminous intestine, which reaches as far as the posterior margin of the body. In the intestine is found a brownish or variegated mass.

Morphologically this species is more closely related to the young encysted larva of the human lung distome found in the crab than

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\*The cercariae which came to my observation have been provisionally called by numbers.

No. 12, which has been reported as the cercaria of the human lung distome in the Journal of Experimental Medicine, vol. 26, No. 3. This new discovery has already been reported in Japanese in the Tokyo-Iji-Shinshi, February, 1918.

Since that date, various workers published their views regarding this species of cercaria. Kobayashi reported in Japanese the results of his study on the cercaria of the human lung distome carried out in Corea in the Chosen (Corean) Igakkai Zasshi No. 21. He thinks that the cercaria A, as he names it, which is found in *Melania gottschei* M., *Melania nodiperdo* var. *quinaria* M. and *Melania extensa* (?) M., is the cercaria of the human lung distome. It seems very likely that this species of cercaria is identical with mine. Besides, Miyairi, who is studying the first intermediate host of the human lung distome, holds

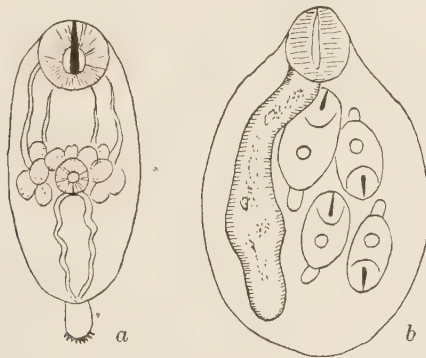


Fig. 1.—Human lung distome; a, cercaria,  $\times 160$ ; b, redia,  $\times 80$ .

the view that this species, which is found in *Melania pannicincta* M. and *Melania extensa* M., must belong to the cercaria of the human lung distome. It was reported also in Japanese by him in the "Ikaijiho," No. 1252, that he had succeeded in obtaining this species experimentally by infecting the water snails with the miracidium of the human lung distome.

Yoshida's cercaria H seems to belong to this species. He states that his cercaria H is provided with the pharynx being situated just in the middle or a little anterior to the middle part of the oral and the abdominal suckers, but in our cercaria it is lacking. Neither are the spines found at the end of the tail. These differences may be due to a difference of observation. Yoshida, however, does not attempt to institute any relation of this species to the cercaria of the human lung distome (Osaka Igakkai Zasshi vol. 16, No. 3).

## II. YOUNG ENCYSTED LARVAE FOUND IN THE CRAB

The young encysted larvae in the crab hitherto supposed to be those of the human lung distome as reported in my former communication in the Journal of Experimental Medicine, vol. 26, No. 3, was since identified by Dr. Yokokawa and myself to belong to an undescribed species of parasite, *Stephanolecithus parvus*. Ever since, I have carried out investigation directed toward the discovery of those of the human lung distome in the crab, and I think I have succeeded in doing this.

This form is chiefly found wedged in the muscular tissues or in the epidermis of the crab. Its shape and size vary according to the ages of the worm.

The youngest specimens move freely through the tissues of the host, showing a squirming motion in a thin cyst wall. The cyst is very



Fig. 2.—Encysted larvae; *a*, youngest found,  $\times 120$ ; *b-e*, successive stages,  $\times 80$ ; *f*, nearly full grown,  $\times 80$ ; *g-h*, full grown,  $\times 80$ .

pliable and changes its shape according to the worm inside. The stretched specimen together with the cyst measures 0.18 to 0.26 mm in length by 0.11 to 0.1 mm. in breadth. The oral sucker is provided with a sharp spine. The abdominal sucker is smaller than the oral. It lies a little anterior to the middle part of the body. The excretory vesicle is slit-like, being situated on the median line arising from the dorsal side of the abdominal sucker or sometimes beyond the sucker and reaches as far as the end of the tail. The intestine has not yet developed.

Some of the encysted larvae have a globular shape, 0.18 to 0.22 mm. in diameter. The larva lies in the thin wall of the cyst, folded on itself. The excretory vesicle is well developed. It reaches a little

anterior beyond the middle part of the body, and has a dark gray color. The suckers are just the same as those just described.

The large specimen has the diameter of 0.24 to 0.26 mm. The wall of the cyst is thin and pliable. The worm lies folded on itself or sometimes straight. The excretory vesicle is very large, and has the content consisting of coarse granules of a gray color. The excretory vesicle is especially thickly provided with pigment. A slender, long and winding intestine lies laterally along the excretory vesicle. It is somewhat difficult to detect it. The encysted larvae are sometimes oblong in shape. The cyst wall is so thin that slight pressure breaks it, liberating the worm.

The young distome just out of the cyst has a leaf-like form 0.3 mm. long by 0.15 mm. wide. The oral sucker has the diameter of 0.04 mm., and is provided with one spine. The pharynx is well developed. The esophagus is short. The intestine is slender and takes a slightly winding course laterally to the excretory vesicle. The abdominal sucker is smaller than the oral, and measures 0.035 mm. in diameter. It is situated a little anterior to the middle part of the body. The surface of the worm is covered with short, weak spines.

The encysted larvae are found both in the muscular tissues and the epidermis of the crab. Usually we come across very few of them. The reason for this is probably that the encysted larvae of this species are not conspicuous and are likely to be overlooked, being taken for the section of the muscular tissues or deposition of the cuticular pigment. The thinness of the wall of the cyst may make its detection very difficult. In one of the crabs kept for two weeks in confinement I found a very large number of the fairly well developed encysted larvae. Outside the muscle and the epidermis, they were also found in the liver, where their detection is most difficult. They have not yet been discovered in the gills.

This species of the encysted larvae appears very different from the full grown encysted larva of the human lung distome, but close examination will reveal that they decidedly show characteristic developmental stages of the human lung distome. I do not hesitate to state that its identity with the larvae of *Paragonimus westermani* is a matter beyond dispute.

## DISSOTREMA SYNONYMOUS WITH GYLIAUCHEN

SEITARO GOTO

Imperial University of Tokyo

In a recently published paper (Goto and Matsudaira, 1918) I described jointly with the late Mr. Matsudaira an amphistomatid parasite as a new genus and species under the name of *Dissotrema papillatum*. A search of some further literature to which I was able to gain access later showed, however, that a worm had been described by Nicoll in 1915 under the name of *Gyliauchen tacharodes*, with which my species presents so many points of close resemblance that the two should be placed in the same genus. *Dissotrema* therefore becomes by the rule of priority a synonym of *Gyliauchen*.

*Gyliauchen tacharodes* was found in considerable numbers in the intestine of a pilot fish (*Tachysurus* n. sp.). The body measured 2.6 to 3.5 mm. in length, was elongated, moderately plump and somewhat pointed at both ends. Cuticula entirely smooth. Oral sucker globular, sub-terminal, 0.24 to 0.26 mm. in diameter; posterior sucker almost at the end of the body, somewhat elongated, 0.50 by 0.47 mm. Prepharynx extraordinarily long, passing down on the right side of the body to the level of the pharynx, where it bends at right angles and passes over to the left side of the body; here it again bends at right angles and proceeds toward the front end of the body, but again bends abruptly backward about midway between the oral sucker and the pharynx and proceeds along the median line of the body to join the pharynx. This extremely muscular organ is 0.28 to 0.35 mm. by 0.23 to 0.27 mm. in size and lies between the first and second third of the body. Intestinal caeca arising directly from the pharynx, short, widely dilated, somewhat horse-shoe shaped, terminating at the level of the ovary, about two fifths of the body length from the posterior end. Genital aperture median, just behind the intestinal bifurcation, some distance in front of the middle of the body; cirrus pouch stout, muscular, ovoid, 0.35 by 0.25 mm. in size, enclosing an almost globular pars prostatica of moderate size and a rather wide ductus; vesicula seminalis L-shaped with the lower limb directed toward the right, immediately following the pars prostatica, considerably larger than the cirrus pouch; testes usually in an oblique pair, a little in front of the acetabulum, usually overlapping a little, almost globular, 0.3 mm. in diameter. Receptaculum seminis globular, nearly as large as the testes, immediately in front of them and in the left half of the body; ovary

much smaller than the receptaculum seminis, immediately in front of it but more toward the median line of the body, usually almost contiguous with the vesicula seminalis. Vitellarium rather scattered and irregular, situated for the most part laterally over the intestinal ceca, not extending beyond the latter posteriorly but anteriorly reaching a little in front of the pharynx; follicles small; yolk ducts running down from the posterior end of the vitellarium on each side, passing round the outer edges of the testes and then turning forward to unite between the testes. Uterus containing very few ova, which are light yellow in color and 78 to 84 by 45 to 49 $\mu$  in size. No statement is made about the excretory or the lymph system.

A comparison of the two species *tacharodes* and *papillatus* enables me to formulate the generic diagnosis more accurately than was possible in my former paper.

Genus *Gyliauchen*. Body plump, lightly attenuated at the front end, broader and more rounded at the hind end. Cuticula smooth. Oral sucker globular or ellipsoidal, close to anterior end; acetabulum subterminal, opening on the ventral surface by a longitudinally elongated aperture. Genital opening in the ventral median line in the middle third of the body. Testes subglobular, in oblique pair, directly in front of acetabulum. Ovary subglobular, some distance in front of the testes, median or submedian, much smaller than the testes. Receptaculum seminis globular or flask shaped, subequal to testes in size or notably smaller. Vitellaria lying for the most part in the anterior half of the body, in small, isolated follicles; paired yolk ducts proceeding backward from the hind end of the vitellaria. Cirrus pouch muscular, ovoid, conspicuous. The whole genital organs, with the exception of the vitellaria, lying for the most part in the posterior half of the body. Prepharynx very long and convoluted, terminated by a muscular pharynx; intestinal ceca short and wide, extending only for a short distance into the posterior half of body. Excretory vesicle elongated bottle-shaped, opening by its slender end near the hind end of body; paired excretory vessels proceeding forward from the antero-lateral corners of the vesicle.

Nicoll does not mention the cirrus glands, nor the prepharyngeal glands, but there is a suggestion of the presence of the former in his figure, while as to the latter there is no doubt in my mind that they will be detected on a closer examination.

Concerning the affinity of *G. tacharodes* Nicoll says that it undoubtedly belongs to the family Paramphistomatidae, but that it is difficult to include it in any of the subdivisions of that family, though most closely, albeit remotely related to the *Pseudocladorchis* of Daday. I

pointed out in my former paper certain resemblances and differences between my parasite and the Paramphistomatidae and concluded that they were such as to make it more a matter of convenience than of principle whether to refer it to that family or a new one, but that the erection of a new family was more desirable. A classification of the Amphistomatidae has been recently attempted by Stunkard, but it is an admittedly provisional one to be replaced by a more natural system when such is proposed. Now there is one aberrant genus of this family to which I ought to have paid more attention when discussing the affinity of my parasite, and that is *Balanorchis* (Fischöeder), the exact rank of which Stunkard leaves undecided. In this genus the testes lie in an asymmetrical pair directly in front of the acetabulum and posterior to the ovary, and what is of equal importance is the presence of a cirrus pouch, although its wall appears to be relatively quite thin. If this parasite lost its buccal pouches and perioral papillae, shortened its intestinal ceca and acquired a pharynx at the termination of its long esophagus a form closely similar to *Gyliauchen* would result. More distantly related to *Gyliauchen* appears to me to be the parasite described by Nicoll immediately before *G. tacharodes* under the name of *Opistholebes amplicoeus*, from the intestine of *Sphaeroides lunaris*, which the author considers to be not an amphistome but an anomalous distome. If this worm had a long, winding prepharynx and shorter intestinal ceca it would not be very unlike *Gyliauchen*, altho we do not know anything at present about its excretory system or its possession of a lymph system. *Opistholebes* appears to be related in turn to Nicoll's *Maculifer subaequiporus* also described in the same paper from the intestine of *Sphaeroides multistriatus*. This is a true distome and related, as Nicoll points out, to the Allocreadiidae; but if it had its acetabulum transferred to near the hind end of the body a worm would result, which is quite like *Opistholebes* except for the muscular ring of the prepharynx, the systematic significance of which it is difficult to evaluate at present. One more aberrant distome I may mention in this connection and that is the species described by Monticelli as *D. fractum* Rudolphi. It has a long, winding prepharynx surrounded in its entire course by numerous unicellular glands which the author calls "salivary," and which are evidently analogous to the prepharyngeal glands of *Gyliauchen papillatus*; in other respects, however, this distome stands quite remote.

These brief observations serve to show that *Gyliauchen* stands intermediate, when single characters are compared, between the Paramphistomatidae and some of the aberrant distomes. That it is sufficiently distinct from the former to justify the erection of a new family has been pointed out.

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## NEW HUMAN PARASITES

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*Dicercomonas* Chalmers and Pekkola, 1919 (not Diesing, 1856). *Dicercomonas soudanensis* Chalmers and Pekkola, 1919.—The proposed new species represents a new genus of flagellates of the family Tetramitidae and is characterized by the absence of cytostome and contractile vacuole, and presence of a simple nucleus, and two anterior and one posterior flagellum, the last being attached to the body for a portion of its length, but ending freely. The species in question was found in fluid feces in a few cases of diarrhea in Khartoum. The zoological position of this flagellate is discussed and a diagram illustrating the relationships of the genera and subfamilies of the Tetramitidae is given, also a key for distinguishing *Dicercomonas* and other genera comprising the subfamily Embadomonadinae. [*Dicercomonas* Chalmers and Pekkola is a homonym of *Dicercomonas* Diesing, 1856, and hence if recognized as a distinct genus must be renamed—B. H. R.] (J. Trop. Med. & Hyg., 22: 29-30; 1 pl., Feb. 15, 1919).

*Ornithodoros maroccanus* Velu, 1919.—This new tick from North Africa is readily distinguishable from *O. erraticus* (Lucas), also a North African species, but is very similar to *O. turicata* (Dugès), an American species. It clearly differs from the latter, however, in certain details of the legs and cuticle. It attacks human beings and pigs, its bite is painful, and gives rise to a pronounced local reaction of the skin which lasts for several days, sometimes accompanied by fever (Bull. Soc. Path. Exot., 12: 99-104, 9 figs.).

*Oncocerca caecutiens* Brumpt, 1919.—This species of nematode from Guatemala, which is described and figured by Brumpt, closely resembles *Oncocerca volzulus*. It occurs in subcutaneous tumors usually located on the head. According to Robles (Bull. Soc. Path. Exot., 12: 442), it is the cause of a disease known as coastal erysipelas. In some localities as many as 97 per cent. of the population may be infested with this nematode, Indians more commonly than white, and children and adult males more commonly than adult females. As a rule, only field laborers are affected. Robles thinks that certain species of *Simulium*, which are common in the localities where the parasite is found, serve as vectors (Bull. Soc. Path. Exot., 12: 464-473; 5 figs.).

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## NOTE

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Hookworm disease is a serious matter in Queensland according to Dr. Lambert, who has been investigating for the Rockefeller Foundation. Not less than 23 per cent. of the population of the coast are infected, and if the progress of the disease is not arrested serious degeneracy may be expected in a few generations. Despite denials from political officers statements in the report are confirmed by abundant evidence from scientific sources.





PLATE V

EXPLANATION OF PLATE

*Notropis anogenus* Forbes bearing cysts of *Myxobolus aureatus* in the fins. The cysts in life correspond in color to Japanese gilding. Drawn from life by Mrs. H. S. Jennings, Put-in-Bay, Ohio, August 15, 1898.